ADVANCES IN INTERNAL MEDICINE

VOLUME III

CONTRIBUTORS TO VOLUME III

E. B. ASTWOOD, M.D., Joseph H. Pratt Diagnostic Hospital, and Department of Medicine, Tufts College Medical School, Boston, Mass.

JEROME W. CONN, M.D., Associate Professor of Internal Medicine, University of Michigan Medical School, and Director, Section on Endocrinology and Metabolism, University Hospital, *Ann Arbor, Mich*

WILLIAM DAMESHEK, M D, Blood Research Laboratory, Joseph H Pratt Diagnostic Hospital, and Department of Medicine, Tufts College Medical School, Boston, Mass

HARRY EAGLE, M D. National Institutes of Health, U S. Public Health Service, Bethesda, Md

SOLOMON ESTREN, M D., Laboratory of Hematology, The Mount Sinai Hospital, and Department of Medicine, New York University College of Medicine, New York, N Y

WILLIAM H. FELDMAN, D V M, M Sc, D Sc, Professor of Comparative Pathology, Mayo Foundation, University of Minnesota, Rochester, Minn.

H. CORWIN HINSHAW, M D, PhD., D.Sc., Clinical Professor of Medicine, Stanford University School of Medicine, San Francisco, Calif

CHARLES HUGGINS, M D, D Sc., Professor of Surgery, *University of Chicago, Chicago, Ill*

CHARLES A. JANEWAY, M D, Thomas Morgan Rotch Professor of Pediatrics, Harvard Medical School, and Physician-in-Chief, Children's Medical Center, Boston, Mass

WARFIELD T LONGCOPE, M D, Cornhill Farm, Lee, Mass, and Emeritus Professor of Medicine Johns Hopkins University, Baltimore, Md

JOHN A. LUETSCHER, JR., M D, Associate Professor of Medicine, Stanford University School of Medicine, San Francisco, Calif

H HOUSTON MERRITT, M D, Columbia University, and Neurological Institute, Presbyterian Hospital, New York, N. Y

HENRY PINKERTON, M D, *Professor of Pathology*, St. Louis University School of Medicine, St Louis, Mo

PAUL TALALAY, M.D, Department of Surgery, Massachusetts General Hospital, Boston, Mass

ADVANCES IN INTERNAL MEDICINE

EDITORS

WILLIAM DOCK, M.D., Long Island College of Medicine, Brooklyn, N. Y.

I. SNAPPER, M.D , The Mount Sinai Hospital, New York, N Y

ASSOCIATE EDITORS

TINSLEY R. HARRISON, M D.
Southwestern Medical College
Dallas, Texas

CHESTER S KEEFER, M.D
Evans Memorial and
Massachusetts Memorial Hospitals
Boston, Mass

WARFIELD T LONGCOPE, M D.
Cornhill Farm
Lee, Mass.

GEORGE R MINOT, M D
Thorndike Memorial Laboratory
Boston City Hospital
Boston, Mass

J MURRAY STEELE, M.D
Goldwater Memorial Hospital
New York University Division
Welfare Island, N Y

VOLUME III

1949

INTERSCIENCE PUBLISHERS, INC., NEW YORK
INTERSCIENCE PUBLISHERS LTD., LONDON

INTERSCIENCE PUBLISHERS, INC
215 Fourth Avenue, New York 3, N Y.

For Great Britain and Northern Ireland
INTERSCIENCE PUBLISHERS LTD
2a Southampton Row, London, W C 1

PRINTED IN THE UNITED STATES OF AMERICA BY
MACK PRINTING CO, EASTON PA.

PREFACE

The editors and publishers of *Advances in Internal Medicine* wish to thank the contributors for exemplary patience in dealing with the exigencies of the printing and bookmaking trades in 1947–1948 Physicians who have struggled with varityped journals, or no journals appearing for months, realize how it happens that an up-to-the-minute contribution on a lively topic may seem out of date and need much revision when publication is delayed. Many of the contributors to this number have experienced such an unfortunate delay and have made extensive revisions in the proofs of their articles. The editors believe that they have succeeded in keeping the contributions coherent, comprehensive, and up to date. For this tiresome task of revision the editors are especially grateful. It is hoped that in the following numbers no such delays will be encountered

W. Dock
I. Snapper

New York, N. Y.
April, 1949

CONTENTS

ADVANCES IN INTERNAL MEDICINE

VOLUME III

Use of British Anti-Lewisite (BAL) in Treatment of Poisoning by Arsenic, Mercury, and Other Metals

Warfield T. Longcope and John A. Luetscher, Jr.
The Medical Clinic of the Johns Hopkins University and Hospital, Baltimore, Maryland

Introduction

The development of 2,3-dimercapto-1-propanol (British anti-lewisite, or BAL) offers an effective antidote against arsenic mercury, and possibly other metallic poisons. The successful action of BAL depends on the formation of such stable BAL–metallic compounds that the metal can be removed from the body in a harmless state. Such an ideal action has been achieved with certain types of poisoning in man, but in other cases BAL may be ineffective Certain fundamental principles govern the toxicity of metallic poisons and their elimination from the body during treatment with BAL, these will be briefly reviewed in order to give a rational basis for treatment The rest of this review will be devoted to experience in the treatment of various types of metallic poisoning with BAL and some closely related compounds.

BAL is the practical result of a series of investigations on a much broader theme. It has now become clear that the effect of the metallic poison is to combine with and inactivate certain vital enzymes of living cells Systematic study of the chemical groups with which arsenic combines in the cell gave the hint leading to the development of a drug that combines even more readily with the metal and that can remove the metallic poison from its destructive attachment to the cell

Two fundamental lines of thought were laid down by Ehrlich in his study of arsenical parasitocidal drugs. Arsenic is not ordinarily a corrosive poison or an active protein precipitant, yet very small quantities of the organic arsenicals kill sensitive cells It was logical, therefore, to assume that the poisonous effects of arsenic were

due to a specific action of the metal on some important chemical component of the cell. Ehrlich visualized the toxic action of arsenic as a chemical combination with the parasite or body cell: "Corpora non agunt nisi fixata" (1) A mass of evidence now supports this view. The toxicity of an organic arsenical depends to a great extent on its fixation in the tissues (2–4). The wide variation in the toxicity of these compounds results from the variation in the proportion of administered arsenic which is bound to the cells The amount of arsenic bound to the cells after a lethal dose of an arsenical drug was found to be very similar for a variety of such drugs (4).

The affinity of sulfhydryl (thiol, —SH) compounds for mercury led to their name, "mercaptans" Arsenic reacts with simple sulfhydryl compounds to form fairly stable compounds called thioarsinites. When sulfhydryl groups were identified in living cells (5), Ehrlich suggested that they might be the "arseno-receptors," the specific chemical groups in the cell with which arsenic combined (6). It was subsequently found that when arsenic acted on cells and proteins, the sulfhydryl groups disappeared (7,8) and the arsenic was bound to the protein (8). These findings represented strong evidence that arsenic combines specifically with the sulfhydryl groups of the tissue proteins

Although some arsenic may combine with nonessential sulfhydryl groups, there are many important enzymes which are dependent on free sulfhydryl groups for their activity and are thus highly sensitive to arsenic and other heavy metals (7,9–13) When such an enzyme system is poisoned by combination with the heavy metal, the cell may be deprived of energy necessary for its function or even for its survival Other chemicals which specifically attack sulfhydryl groups also inactivate enzymes sensitive to the metallic poisons (11,14–16). Certain organic mercurials are highly specific sulfhydryl inactivators

It became clear from these studies that the binding of sulfhydryl groups of proteins and enzymes is a specific cause of the toxicity of arsenic. The defense or liberation of these groups from the toxic combination was indicated, and the investigator naturally turned to simpler sulfhydryl compounds as competitors for arsenic Voegtlin, Dyer, and Leonard (9) demonstrated excellent, but temporary, protection of animals when cysteine, glutathione, or sodium thio-

glycolate, thiolactate, or thiosalicylate was administered before a lethal dose of arsenic was given. All of the animals subsequently died, however, and there was even some evidence of an increase in the ultimate toxicity of the arsenical. Very large doses of the protective agent were necessary. The duration of protection was about the same as the persistence of the sulfhydryl compound in the circulating blood. Voegtlin pointed out that the thioarsinites formed by these simple sulfhydryl compounds must be unstable, and that protection thus failed unless an excess of the sulfhydryl compound was present.

Further experience has amply confirmed Voegtlin's conclusions. Walker (7) found that motile protozoa immobilized by an arsenical could be revived on the addition of sulfhydryl compounds, only to die some hours later. Labes (17) reported temporary protection of mice poisoned with arsenious acid when cysteine was given. Cohen, King, and Strangeways (18,19) studied a number of thioarsinites for chemical and pharmacologic properties. They found that the thioarsinites were unstable unless an excess of sulfhydryl groups were present. The toxicity of a thioarsinite depended on the free arsenic released from the thioarsinite by dissociation of the complex. Such a release of arsenic could be reduced by an excess of sulfhydryl compounds. During the course of this study, ethanedithiol, a close relative of BAL, was examined, but the authors did not comment on its stability, which might have offered the key to the problem of arsenical toxicity.

In spite of the failure of the simple monothiols in the treatment of arsenical poisoning, interest was aroused by their temporary protective action. Further encouragement came from the success of these compounds in the defense and reactivation of enzymes from poisoning by arsenic and by other poisons which attack sulfhydryl groups (11,14–16). Eagle found that monothiols in high concentrations would protect spirochetes not only against arsenic but against mercury and bismuth as well (20).

The problem was now clearly defined, but the solution was elusive. A number of simple monothiols could defend the vital tissue thiol groups against arsenic, but failed to protect animals because they were not stable enough to hold the arsenic until it could be excreted. What was needed was a nontoxic substance which would form a stable and readily excreted thioarsinite.

An intensive search for an antidote against arsenical war gases was begun in 1939 in the laboratories of Professor R. A. Peters at Oxford (24). The specific action of arsenic on pyruvate oxidase, an important enzyme system of the brain, had already been established (12). This is the same enzyme system which fails when a deficiency of thiamine deprives it of its essential "cocarboxylase" component. The protein component of the enzyme contains an essential sulfhydryl group, which is sensitive to traces of arsenical compounds and to a number of other sulfhydryl poisons (12,16) This enzyme was used as an *in vitro* test for the toxicity of arsenical compounds and for the evaluation of antidotes (23,44). The results closely paralleled the previous experience in animals. The available monothiols and dithiols failed to protect the enzyme system against highly toxic arsenicals (23), and the instability of the thioarsinites was again demonstrated (24,30).

The problem was solved by a brilliant inference from the observation that arsenic combined with two thiol groups in keratein, a protein derived from skin (21). Since this compound was very stable, Stocken and Thompson reasoned that a cyclical thioarsinite was formed, and that simple organic compounds with two closely spaced thiol groups might also form similar stable compounds When further evidence confirmed this hypothesis (22), a search was begun for a compound of this chemical class with suitable biologic properties. The third compound studied was 2,3-dimercapto-1-propanol (BAL), which was found to be effective, reasonably nontoxic, well absorbed through the skin, and rapidly excreted in the urine (23–26).

BAL and several closely related compounds proved to have such a high affinity for arsenic that it was possible to reverse virtually all of the acute biologic effects of arsenical toxicity Enzymes poisoned with arsenic could be reactivated (23,27,44). Isolated cells could be revived after a lethal dose of arsenic had stopped their motility and given them the appearance of dead cells (25,28) It could be shown that arsenic had actually been removed from such cells when BAL was added to the liquid medium (28) When BAL was applied to the skin after contamination with lewisite (dichloro(2-chlorovinyl)arsine), the toxic arsenical could be removed from the skin, with consequent prevention of much of the local reaction. The excretion of arsenic in the urine of such animals

was greatly increased (29). These experiments will be described in greater detail in the consideration of the treatment of arsenical poisoning; here it will suffice to say that all of the evidence favored the concept that the toxic combination of arsenic with the tissues could be prevented or reversed.

A point of therapeutic significance emerged from these studies. The proportion of cells which could be revived by BAL after arsenical poisoning decreased rapidly as the time between poisoning and treatment was lengthened (28). Arsenic might still be removed from the cells, and enzymes might be reactivated, but the cells failed to recover if too long an interval of poisoning had preceded the addition of the antidote. Certain clinical instances of chemical success (increased excretion of the poisonous metal) and biologic failure of BAL may be explained on this basis. Prompt treatment is the most effective treatment.

Important information was also gained from the study of the toxicity of the complexes formed by BAL with metals. Complexes of BAL with highly toxic arsenicals are generally less toxic than the arsenical itself (23), but the compounds of BAL with less toxic arsenical drugs (30) and with other metals (31,32) may be more toxic than the metal alone This apparent paradox is probably due to the dissociation of less stable complexes within the body, and to the release of the toxic metal, perhaps in a vital spot or in a concentration which would not have been reached by administration of the metal alone. In the case of the arsenicals and of mercury, the complexes can be stabilized by an excess of BAL, and their toxicity can be thus abolished (30,31). The therapeutic indication is to give enough BAL sufficiently often to maintain an excess of the dithiol With other metals, however, the dissociation of the BAL–metallic complex may occur at a site where an excess of BAL is ineffectual (32). In such cases, BAL may increase the toxicity of the metal. This possibilty must be taken into account whenever BAL is used in new types of poisoning

A number of metals are known to combine chemically with BAL (24,33). A survey of the effect of BAL on poisoning *in animals* indicates that BAL reduces the mortality rate after severe poisoning by arsenic (23,28,29,44–49), mercury (31,45,50,51), antimony (45, 50,52), cadmium (32,53), bismuth (50), chromium (50), and nickel (50). BAL is ineffective in thallium poisoning (50) and argyria

(54), and increases the mortality of acute and chronic poisoning by lead and selenium (50). These findings must not be accepted as final conclusions, since effectiveness or failure may depend on the method of poisoning or treatment When BAL is found to be harmful in experimental poisoning, however, there is a strong probability that untoward effects will be produced in man

The rapid destruction and excretion of BAL in the body (26,29, 33) limits its therapeutic and its toxic effects to a few hours after each injection. Eagle found that in both animals and man the increased excretion of arsenic in the urine lasted for 2 to 4 hours after administration of BAL (28,34). Similarly, toxic effects of BAL have been found to last only an hour or two, and there is little or no cumulative toxicity if injections are spaced at intervals of 3 or 4 hours (28,35–37) It is generally considered that administration of BAL every 4 hours is safe and effective The interval between injections may be reduced to 3 hours when it is necessary to maintain a high concentration of circulating dithiol Longer intervals are permissable when the acute phase of poisoning has passed.

Toxic symptoms due to BAL may occur when large doses are given (35,36,37), but no serious or lasting ill effects have been observed in over 400 cases treated with the drug In animals, the minimal lethal dose of BAL is 10 to 25 times the usual therapeutic dose in man, on a basis of body weight (23,28,38,39) The minimal lethal doses for several species are of the same order of magnitude, although some of the published data reflect the higher toxicity of earlier preparations of BAL The dose–mortality curves are quite steep, indicating that idiosyncrasy to BAL is uncommon These observations make it probable that there is a considerable margin of safety at the usual dosages in man

When animals are given several times the usual maximal human dosage, they exhibit apathy, lacrimation, blepharospasm, salivation, and retching (38,39). Hyperpnea, hypertension due to peripheral vasoconstriction, albuminuria, and electrocardiographic changes may occur at somewhat larger doses (29,38–41) As lethal doses are approached, muscle tremors, incoordination, nystagmus, convulsions, and coma appear Respiration becomes labored, pulmonary edema and pleural effusion may occur The early rise in blood pressure gives way to circulatory collapse with hemoconcentration

Death occurs within a few hours after lethal doses, while surviving animals recover completely in an equally brief time. There are virtually no morphologic changes after a fatal dose of BAL. Congestion of viscera and fluid in the serous cavities or lungs are the usual findings.

There are striking biochemical signs of generalized poisoning in animals receiving lethal amounts of BAL (38,39). The carbon dioxide content and carbon dioxide combining power of serum are reduced. The *p*H of the blood falls and the concentration of sodium is reduced. Lactic acid concentration in the plasma rises The blood sugar may rise initially and then falls to hypoglycemic levels Terminally, the amino acid concentration of the serum increases. The liver is depleted of glycogen and potassium, while the sodium and chloride content is increased Muscle glycogen is little affected. Many of these changes are similar to those observed in hemorrhagic shock, but occur more promptly after BAL poisoning.

The poisoning of important enzymes by excessive amounts of BAL probably accounts for the profound biochemical disturbance. High concentrations of BAL inactivate a number of enzymes which have an essential metallic component (42,43). An exception is the cytochrome system, which can oxidize BAL without injury, though there may be some temporary interference with its normal function. Insulin is inactivated on contact with BAL *in vitro*, but the relationship *in vivo* is not entirely clear (38,43) Methemoglobin is reduced to hemoglobin by dithiols, and hemoglobin itself may be destroyed by dithiols under certain conditions (33). These changes presumably do not occur except at high concentrations of BAL.

Since the chemical structure necessary for antidotal activity consists simply of two sulfhydryl groups on adjacent carbon atoms, the number of possible analogues of BAL is infinite. A group of dithiols were prepared by Stocken and Thompson (23), ranging from the simplest possible analogue, ethane dithiol, to derivatives of more complex organic compounds. All of the compounds tested were highly active *in vitro*, but when these compounds were tested in animals, the situation was more complicated In a study of compounds related to BAL, Chenoweth, Modell, and Riker (55) found that the toxicity of a variety of these compounds was not very different when calculated in terms of moles rather than absolute weight There was some variability in the speed and in the site of

their toxic action. Several compounds caused more pronounced effusions into the lungs, pleura, and pericardium Chronic injury to the central nervous system was produced by one group of compounds. Most of the simple dithiols resembled BAL in the brief duration of action and in the absence of anatomic pathology after fatal intoxication. Fitzhugh *et al.* (56) compared the relative effectiveness and toxicity of a series of analogues of BAL They found that only a few of the group shared the efficiency and safety of BAL, and that none was definitely better for protection against toxic arsenicals. Because of the technical difficulty of preparing the dithiols and because no compound was found to be superior to BAL as an antidote against lewisite, only small amounts of the other dithiols were prepared, while BAL is available in considerable amounts. Several of the other dithiols deserve further attention, and may ultimately prove to be superior to BAL in certain cases

The glucoside of BAL has certain important advantages. Danielli and co-workers (57) prepared a glucoside of remarkably low toxicity and high activity. This preparation is stated to be about one-hundredth as toxic as BAL, presumably because of its failure to penetrate into cells and because of its rapid excretion by the kidneys. It is effective against intoxication by lewisite. An interesting point was the superiority of the combination of BAL with its glucoside over either antidote alone in the late treatment of lewisite poisoning. McCance and Widdowson (58) administered the glucoside intravenously in man, and described a considerable increase in the urinary excretion of copper and zinc, with little effect on the urinary excretion of iron McDonald (59) noted an increased excretion of copper by sheep after administration of the glucoside. The British group, working with the glucoside, was greatly impressed by the low toxicity of the glucoside Gilman and co-workers (31) found the glucoside superior to BAL in the treatment of mercuric chloride poisoning in dogs and rabbits. The glucoside is effective in cadmium poisoning, and does not produce the renal injury characteristic of the BAL–cadmium complex (32); but large doses of the glucoside may combine with cadmium to injure the central nervous system in a manner different from either cadmium or the glucoside alone in similar dosage.

Several factors which have kept the glucoside from becoming generally available in this country are the difficulty of obtaining a

good preparation, the instability of the product, and the current practice of converting the barium salt to the sodium salt just before administration. Other related compounds are being studied at present.

Arsenic Poisoning

PROTECTIVE EFFECT OF BAL IN SYSTEMIC ARSENIC POISONING IN ANIMALS

As a phase of their experimental investigations, the British workers, Fell and Allsop (60,61), studied the toxic effect of lewisite and lewisite oxide on living cells *in vitro*, and the therapeutic action of BAL on these tissue cultures. Arsenicals applied in several forms proved highly destructive to the cells growing *in vitro*, but BAL in very low concentrations protected the tissue cultures against the toxic action of these arsenicals It was also determined that tissue cultures poisoned by a just sublethal concentration of lewisite largely recovered when treated with BAL. Evidence was thus furnished to show that BAL actually extracted arsenic from the injured cells

In line with these investigations, ample proof was soon collected to demonstrate that the local application of BAL to the skin of animals and of man afforded not only protection against the local toxic action of lewisite, but had actually a therapeutic effect when employed 15 to 30 minutes after the skin had been contaminated by lewisite (23–26). From these observations it appeared that BAL was capable of penetrating the skin, of combining with the arsenical which had already entered the cells, and of binding it in such a manner that it was extracted from the cells, thus neutralizing the toxic action of the arsenical.

Analogous results were obtained by Mann (62) and by Hughes (63) in the experiments on the treatment with BAL of lewisite injuries of the eyes of rabbits. Lewisite hydrolyzes immediately on contact with the moist surface of the rabbit's eye, producing an arsine oxide and hydrochloric acid. Within a few minutes after exposure to lewisite, histologic evidence of damage appears in all tissues of the anterior ocular segment, indicating deep penetration and rapid necrotizing action of the toxic arsenical The rate of penetration of arsine oxide is very rapid, for 2 to 4 minutes after lewisite is instilled into the eye and the lid is closed little or no

their toxic action. Several compounds caused more pronounced effusions into the lungs, pleura, and pericardium Chronic injury to the central nervous system was produced by one group of compounds. Most of the simple dithiols resembled BAL in the brief duration of action and in the absence of anatomic pathology after fatal intoxication. Fitzhugh *et al* (56) compared the relative effectiveness and toxicity of a series of analogues of BAL. They found that only a few of the group shared the efficiency and safety of BAL, and that none was definitely better for protection against toxic arsenicals. Because of the technical difficulty of preparing the dithiols and because no compound was found to be superior to BAL as an antidote against lewisite, only small amounts of the other dithiols were prepared, while BAL is available in considerable amounts. Several of the other dithiols deserve further attention, and may ultimately prove to be superior to BAL in certain cases

The glucoside of BAL has certain important advantages. Danielli and co-workers (57) prepared a glucoside of remarkably low toxicity and high activity. This preparation is stated to be about one-hundredth as toxic as BAL, presumably because of its failure to penetrate into cells and because of its rapid excretion by the kidneys. It is effective against intoxication by lewisite An interesting point was the superiority of the combination of BAL with its glucoside over either antidote alone in the late treatment of lewisite poisoning. McCance and Widdowson (58) administered the glucoside intravenously in man, and described a considerable increase in the urinary excretion of copper and zinc, with little effect on the urinary excretion of iron McDonald (59) noted an increased excretion of copper by sheep after administration of the glucoside. The British group, working with the glucoside, was greatly impressed by the low toxicity of the glucoside Gilman and co-workers (31) found the glucoside superior to BAL in the treatment of mercuric chloride poisoning in dogs and rabbits The glucoside is effective in cadmium poisoning, and does not produce the renal injury characteristic of the BAL–cadmium complex (32); but large doses of the glucoside may combine with cadmium to injure the central nervous system in a manner different from either cadmium or the glucoside alone in similar dosage.

Several factors which have kept the glucoside from becoming generally available in this country are the difficulty of obtaining a

combat both the local and the systemic effects of arsenical intoxication was well brought out by the investigations of Stocken and Thompson (23).

They demonstrated that systemic intoxication of rats following intramuscular injections of sodium arsenite could be prevented by intraperitoneal injections of BAL in aqueous solution, made after an interval of 20 minutes, and that 2 subcutaneous injections of BAL instituted 2 hours after the production of lewisite skin burns gave 100 per cent protection against death from systemic intoxication.

Stocken, Thompson, and Whittaker (44), extending these investigations to other arsenicals, showed that BAL dissolved in propylene glycol saved all rats when it was injected in doses of 40 to 50 mg. per kilogram of body weight from 5 to 7 minutes after lethal doses of mapharsen and neoarsphenamine had been injected intramuscularly Marked signs of generalized intoxication are observed in rats within 15 minutes after the intramuscular injection of mapharsen, but even at this time about two-thirds of the rats survived if BAL was injected intramuscularly. Similar results were obtained by the Canadian group (26)

At this time, intensive investigations upon the therapeutic effect of BAL on arsenical intoxication were being carried out in the United States as well as in England Eagle and associates (28) had already shown that trypanosomes immobilized and to all intents and purposes "killed" by applications of arsenicals could be resuscitated by the addition of BAL, which removed the arsenical from the cell They demonstrated that rabbits injected intravenously with a single, large dose of mapharsen (20 mg /Kg.) could be saved if BAL in propylene glycol solution was injected intramuscularly or intravenously 5 minutes after the arsenic Doses of the same amount of mapharsen were invariably fatal to control animals In tests that were even more rigid, rabbits received 8 mg of mapharsen per kilogram of body weight at hourly intervals for 4 hours. With this dosage, 15 per cent of the animals had died before treatment with BAL was started, and many of the remaining animals seemed to be moribund In spite of this intensive intoxication, 40 per cent of the living animals were saved by injections of BAL

Confirmatory evidence of the therapeutic action of BAL against mapharsen poisoning was obtained in cats by Riker (48); in dogs

toxic material remains on the surface of the cornea. Hughes (63) found that arsenic penetrated into the anterior chamber within $1\,^1/_2$ minutes. It was obvious, therefore, that surface decontamination was of little avail, and that for therapeutic purposes an agent would have to be employed that had the property of penetrating the tissues.

Hughes employed an ointment containing either 5 or 10 per cent BAL in a base consisting of 5 per cent benzylbenzoate (in which BAL is soluble), 20 per cent peanut oil, 20 per cent absorbent base, 10 per cent cetyl alcohol, 14 per cent glycerine monostearate, and 21 per cent liquid white petrolatum. A solution of BAL in propylene glycol was also employed. Preparations containing BAL in concentrations greater than 10 per cent were apt to be irritating, and the maximal beneficial effect was obtained with 5 per cent BAL in ointment or solution. Mann and associates (62) employed 5 to 20 per cent solutions of BAL

The instillation of BAL solution or ointment, within 2 minutes after the end of exposure to lewisite, usually prevented the appearance of any corneal reaction. Treatment after 5 minutes was followed by a transient conjunctivitis and corneal reaction lasting a few days. Treatment after 10 minutes was less effective, since a moderate or mild corneal opacity persisted for 7 days (63), though complete recovery usually took place When 30 minutes had elapsed before starting treatment with BAL, the severity of the ocular lesion was lessened, compared to the controls, but permanent damage to the eye remained It was found that BAL facilitates the disappearance of arsenic from the tissues of the eye, probably competing favorably with arsenic reversibly bound to the tissue components

These observations upon the local effect of BAL upon the skin and on the eyes of animals in preventing and controlling the toxic action of arsenicals furnished ample proof that 2,3-dimercaptopropanol penetrated these tissues and formed a stable combination with lewisite. However, it was shown by the Canadian workers (26) that BAL penetrated the rat's skin rather slowly. The rate at which this occurred was determined by Simpson and Young (26) by incorporating radioactive sulfur in the BAL molecule When BAL was injected intramuscularly, on the other hand, dissemination was wide and rapid.

The practical importance of parenteral injections of BAL to

of the National Research Council. With this background, it was decided to investigate the effect of BAL in the treatment of localized and systemic arsenical intoxication in man. During the early stages of these studies the only available preparations for therapeutic use were ointments suitable only for local application, but after the solution of BAL in benzyl benzoate and peanut oil (28) was ready for distribution the possibilities for more effective treatment of systemic arsenical intoxications were in hand.

Since pharmacologic and toxicologic studies had shown that BAL in concentrated form was toxic for several species of animals, it became necessary to determine the dose of BAL in oil solution that could be safely employed in man

Sulzberger, Baer, and Kanof (36) found that 3 Gm. of 10 per cent BAL in K-Y jelly, rubbed into the skin of the arms and forearms of healthy subjects, caused transitory erythema and urticaria, but no systemic ill effects were observed When 1 ounce of 5 per cent BAL in K-Y jelly was rubbed on the back and shoulders, the response of the skin varied from severe generalized whealing over the entire area of inunction to complete lack of any reaction. Dizziness and faintness occurred in one of the individuals, but there was some doubt as to whether this could be attributed to BAL absorbed from the skin On the other hand, solutions of 10 per cent BAL in 20 per cent benzyl benzoate injected intramuscularly in normal volunteers produced varying degrees of pain at the site of injection, and when doses above a certain amount were employed they gave rise to generalized symptoms. As a rule, increasing amounts of BAL were given every 4 hours for 4 doses Unequivocal complaints generally began at the dose of 250 mg of BAL, or approximately 3 6 mg per kilogram of body weight Symptoms usually appeared within a few minutes after the injection, and reached a maximum in 10 to 30 minutes

The manifestations listed by Sulzberger, Baer, and Kanof in the order of their frequency are: (*1*) nausea, often with vomiting; (*2*) headache, (*3*) burning sensation of lips, mouth, and throat; constricted feeling and sometimes pain in throat, chest, and hands, (*4*) conjunctivitis, tearing, rhinorrhea, and salivation; (*5*) tingling of the hands; (*6*) burning sensation of penis; (*7*) sweating of forehead and hands, (*8*) abdominal pain; (*9*) tremors and shakiness, (*10*) lower back pain, (*11*) elevation of blood pressure. These

poisoned by inhalation of lewisite and phenyl dichlorarsine by Harrison and co-workers (46); and by the same group (47), in mice and dogs suffering from the systemic toxic effects of skin contamination with lewisite and phenyl dichlorarsine In the case of skin contamination in animals, it was necessary to apply BAL to the local skin lesion as well as to inject it in oil subcutaneously or intramuscularly to obtain the most satisfactory results. Absorption of BAL from local applications was not sufficient to combat the systemic effects, while the efficacy of the subcutaneous and intramuscular injections of BAL in oil were much enhanced by local applications, which prevented the continued absorption of lewisite

Further evidence of the detoxifying action of BAL was obtained in the increased excretion of arsenic which Stocken and Thompson (29) and the Canadian workers (26) found to occur in rats after the use of BAL, and which Eagle and associates (28) observed in their experiment with rabbits.

PREPARATIONS OF BAL

In the early experiments, BAL for local application was used in liquid form or in ointments such as that employed by Hughes (63) and in a solution of propylene glycol for injections, since BAL is unstable in water Propylene glycol, however, proved to be an undesirable solvent, for the solution was irritating and deteriorated with time. Eagle (28) found that BAL was soluble in peanut oil, which was a suitable vehicle, when 2 parts of benzyl benzoate for each part of BAL were mixed with the peanut oil. Solutions such as this were stable, could be sterilized, and produced very little irritation on injection A preparation of BAL, which can be employed for intramuscular injection and is now commercially available,* consists of 5 or 10 per cent BAL in 20 per cent benzyl benzoate in peanut oil put up in sealed ampules

DOSAGE IN MAN

The information acquired from the British workers on BAL was made available through the British classified reports, and the results of researches in this country were circulated by means of confidential and restricted reports of the Committee on Medical Research

* Distributed by Hynson, Westcott & Dunning, Inc, Baltimore, Md

of exposure, but in the majority of individuals this region of the skin proved much more sensitive than the skin of other parts of the body.

Although many of the detailed studies which have been recorded in this review had not been made or were only under way when the first therapeutic trials of the effect of BAL upon systemic arsenical intoxication in man were instituted, there was sufficient information to warrant such a procedure on a tentative basis. The work was started by three groups of investigators. Carleton, Peters, Stocken, Thompson, and Williams (65) in England, and Eagle and Magnuson (35) and Longcope, Luetscher, and Wintrobe (66) in this country.

Among the first patients to be treated were 7 women suffering from contact dermatitis caused by diphenylamine chloroarsine (10-chloro-5,10-dihydrophenarsazine), known as adamsite or DM. The dermatitis had persisted for 18 to 50 days, for it does not respond to the usual forms of treatment. BAL in 5 or 10 per cent ointment was applied daily to the weeping, vesicular, erythematous skin lesions in amounts of 100 to 500 mg The inunctions gave rapid relief of itching and burning, but proved exquisitely painful Inunctions were therefore made to the unaffected skin, these proved almost equally effective and resulted in complete clearing of the eruption in 2 to 8 days These tentative trials then led to the employment of BAL in the treatment of arsenical dermatitis, a complication of syphilis therapy with a number of arsenic preparations At first, BAL ointment was used (65,66), but as soon as the 5 per cent and later the 10 per cent solutions in benzyl benzoate and peanut oil became available, these were adopted (65,66), and given by intramuscular injection Doses of 1 5 to 2 cc of these solutions were employed, representing from 100 mg. to 200 mg of BAL Treatment was at first instituted cautiously and patients received comparatively small amounts of BAL, but later the schedule of dosage finally advocated by Eagle (35) was usually followed This consisted in the intramuscular injection of 2 5 mg to 3 mg of BAL per kilogram of body weight, repeated 4 to 6 times daily for the first 2 days, with injections once or twice daily thereafter until recovery

Carleton and co-workers (65) have reported on the treatment of 30 cases of arsenical dermatitis, 9 by inunctions of BAL and 21

signs and symptoms subsided rapidly and there were no lasting effects.

When doses of as much as 5 mg. per kilogram were administered at 4 hour intervals, there was no indication of a cumulative effect, but when the intervals were shortened to 2 hours, the second dose produced a much greater effect than the first

Modell, Gold, and Cattell (37) studied the effects of single intramuscular injections of BAL in 10 per cent solution in doses varying from 3 to 8 mg. per kilogram of body weight in human subjects with secondary and tertiary syphilis In addition to the symptoms already listed their patients became apprehensive and restless, and complained of weakness and fatigue. These authors also stressed an acceleration of the pulse rate and the frequent elevation of blood pressure. All of these symptoms subsided within an hour or two, and doses as large as 5 mg per kilogram could be given at intervals of 3 hours without evidence of a cumulative effect. They found that the minimal dose to produce toxic effects lay between 3 and 5 mg per kilogram. Marked symptoms followed a single dose of 8 mg per kilogram.

Eagle and Magnuson (35) found that toxic symptoms rarely occurred when BAL was used for therapeutic purposes in human beings with arsenical intoxication until a dose of 4 mg per kilogram was reached; but at a dose of 5 mg., two-thirds of the subjects showed symptoms of one sort or another They concluded that doses of 2 5 mg per kilogram could be employed every 4 hours with impunity, and that doses of even 4 mg are feasible, though they may give rise to some symptoms

Though repeated intramuscular injections of 5 and 10 per cent BAL in benzyl benzoate and peanut oil have not appeared to produce general sensitization of the individual to BAL, repeated cutaneous applications of ointment lead to local sensitivity of the skin Sulzberger, Baer, and Kanof (64) investigated this problem in normal volunteers and found that 5 per cent BAL in various bases applied repeatedly to the healthy skin resulted in sensitization to further local inunctions in 16 out of 88 individuals. When, however, the BAL ointments were rubbed into areas of skin that had been previously damaged by chemical irritants, sensitization was much more frequent and was observed in 35 of 53 human subjects The sensitivity produced by BAL was not strictly confined to the area

In a later publication Carleton, Peters and Thompson (651) describe the results obtained in the treatment of an additional series of 44 cases of arsenical dermatitis with BAL. Forty one of these were of the acute exfoliative type Three patients died of complications unrelated to the treatment, while in 4 it was impossible to assess the progress numerically. In the remaining 37 cases, the mean number of days between the first injection of BAL and healing or practically complete healing of the dermatitis was 21.5 days, ranging from 6 to 46 days Abscesses at the site of injection developed in 8 cases



Fig 1 Extensive exfoliative dermatitis involving the face in particular, following administration of 0.3 Gm of diarsenol to a 20 year old Negro See Figure 2 for comparison

Eagle (35) considered that 38 of the patients whose records he analyzed suffered from a mild form of dermatitis. Healing took

by intramuscular injections. Eagle and Magnuson (35) record 88 cases of arsenical dermatitis treated by intramuscular injections, and Longcope and co-workers (66) described the results in 15 patients, 4 of whom were treated by inunction and 11 by intramuscular injections.

It is impossible, for many reasons, to analyze accurately the results of treatment in these three groups of patients. In the first place, there are no extensive and reliable statistics giving the outcome and duration of arsenical dermatitis in patients treated by other methods which could serve as a control for the studies on BAL; in the second place, dermatitis followed the use of several different arsenicals, though the majority of the patients in one group (65) and 10 of the 15 cases in another (66) had been treated with neoarsphenamine; and in the third place, the duration of dermatitis before treatment and its intensity at the time treatment was started differed considerably in individual patients. Other variables such as the amount of the arsenical employed in the treatment of syphilis, the occurrence of dermatitis in hypersensitive patients after small doses, the presence of secondary infections, particularly of the skin, and the variation of intensity and duration of treatment by BAL, complicate still further any final judgment as to the importance of the effect of BAL on the course of arsenical dermatitis.

In spite of these difficulties, the impression produced upon the investigators, which is substantiated by the recorded data, leaves little doubt that BAL is distinctly beneficial in the treatment of arsenical dermatitis All observers agree that within 24 to 48 hours after treatment is started in severe cases, the erythema, edema, and itching often show improvement, while the weeping vesiculitis becomes dryer. In the early stages, scaling may set in, and in the more advanced cases, exfoliation may actually increase Occasionally, progressive recovery takes place with surprising rapidity, but usually, even when the course is favorable, it is some days or weeks before healing of the skin is complete Of the cases reported by Carleton and Peters (65), and treated by intramuscular injections, 18 were severe; 2 patients died, and in the remaining 16 patients the dermatitis persisted for 5 to 90 days. The skin was "almost normal" in a mean of 18 9 days and entirely normal in a mean of 27.6 days.

after treatment was started; 3 patients died in a relapse of the dermatitis after an initial improvement.

In the small group reported by Longcope and associates (66), 4 were treated by inunction and 11 by intramuscular injections. All of the patients suffered from a severe form of exfoliative dermatitis, which in all but 2 was generalized; 12 of the 15 were febrile. Recovery occurred in from 6 to 80 days; 5 patients were completely well within 12 days, while in 4 cases the disease lasted longer than 3 weeks (Figs. 1 and 2). Relapses were observed in 5 patients, and were controlled by reinstitution of BAL therapy.

When the results obtained by treatment with BAL in these groups of patients are compared with the available meager control statistics, the outcome following BAL therapy appears in a very favorable light. Carleton and Peters (65) quote figures for 142 patients, in whom the average time for cure was 55 to 89 days, and in another series without knowledge of the total number of patients, 46 days. From the records collected at the Johns Hopkins Hospital (66), 37 cases of arsenical dermatitis had complicated the administration of 3 or more injections of an arsenical. The dermatitis persisted in this group for 20 to 240 days. In 26 cases, or over 70 per cent, the dermatitis lasted for 40 to 70 days, and the average duration of the entire group was 67.3 days There were 3 deaths among these controls

The tentative conclusions that so far have been expressed (67), and that appear to be upheld by the published data, are that BAL is a valuable drug for the treatment of most cases of arsenical dermatitis, and in some cases causes rapid healing of the cutaneous lesion It seems evident to those who have worked with BAL that more extended experience in its use will bring even better results. Many of the first patients who were treated by BAL were not given large enough doses, and were not treated over a sufficiently long period It was repeatedly noted that relapses occurred, particularly after short courses of BAL, but that these relapses could again be controlled by instituting a second or even a third course of BAL. It has been suggested (65) that relapses may be occasioned by the delayed liberation of arsenic stored in tissues other than the skin. This arsenic might again injure the feeble cells of the skin that were in the process of recovering from the injury caused by the

place in 34 of these in from 1 to 5 days On the other hand, 51 of the patients suffered from a severe variety of exfoliative dermatitis, for many of them had edema of the skin and fever, and



Fig 2 Same patient as in Figure 1, four days later, following inunctions of 1 Gm of BAL in ointment

showed signs and symptoms indicative of a systemic intoxication Improvement took place rapidly in 40 patients, so that many were 70 to 90 per cent recovered 5 days after BAL therapy had been instituted and the majority had "recovered" by the fifteenth day

prompt and vigorous treatment might have been inferred from the previous animal experiments, and was also found to be true in BAL therapy of mercurial poisoning (page 34). Thus, in 24 patients suffering from hemorrhagic encephalitis, who received BAL within 6 hours after the onset of symptoms, 75 per cent recovered; while of 9 patients in whom the first injection was delayed for 9 hours or longer only 4, or 45 per cent, survived. Of the last 7 cases, treated early with intramuscular injections of BAL, 6 survived, giving an over-all recovery rate of 80 per cent for the 31 seriously ill patients who received treatment

The number of patients suffering from blood dyscrasias arising during arsenical therapy is still too small to admit of analysis. Eagle and Magnuson (35) record 11 cases of agranulocytosis, 10 of whom recovered after treatment with BAL. Carleton and associates (65) allude to 5 patients, all of whom recovered, and we have observed 1 patient who recovered during treatment with BAL (68).

Eagle and Magnuson (35) state that BAL had no effect upon 3 patients with aplastic anemia, but we have had 1 patient with thrombocytopenic purpura who recovered promptly on BAL therapy (68). It will be necessary to study many more patients in this group before any conclusions can be drawn as to the value of BAL in the treatment of blood dyscrasias arising during the course of arsenical intoxication.

BAL appears to be efficacious in preventing serious toxic complications occasioned by the accidental administration of massive doses of arsenicals, and in controlling febrile reactions occurring as a complication of arsenotherapy (35).

The results obtained in the treatment of so-called arsenical jaundice have been very disappointing. Eagle and Magnuson (35) reported some improvement in 5 of 14 cases, but Carleton and coworkers (65), and we, ourselves (68), are of the opinion that BAL had no material effect upon the course of the illness in the limited number of cases which have been studied. Indeed, it has been suggested (65), and seems possible, that many of these patients were suffering from epidemic hepatitis, and that their jaundice was not directly related to arsenotherapy.

An important indication that BAL extracts arsenic from the tissues was detected in the experiments of Stocken and Thompson (29), in those of Eagle, Magnuson, and Fleischman (28), and by

arsenic which had recently been removed from them. This hypothesis is substantiated by the *continued excretion of arsenic after* cessation of BAL treatment.

Another factor which appears to prolong the illness and has been responsible for the death of a few patients, is secondary infection. This is common in the skin, may affect the lymph nodes, and has occasionally given rise to abscess formation at the site of injection of BAL, especially in the buttocks. It has often been found necessary to use sulfonamides or penicillin to control these infections. *Pneumonia has occurred as a secondary infection in a small proportion of cases*

There has been some experience to show that BAL may be an effective treatment in forms of arsenical poisoning other than dermatitis. Eagle and associates (35) record the results of their collected statistics in the treatment by BAL of the following conditions complicating the arsenotherapy of syphilis.

Complication	Number of cases
Arsenical dermatitis	88
Hemorrhagic encephalitis	55
Blood dyscrasias	15
Jaundice	16
Accidental administration of massive doses of arsenic	4
Fever, often with associated toxic rash	44
Miscellaneous	5
Total	*227*

Exclusive of the cases of dermatitis, the largest group of patients treated with BAL were those suffering from encephalitis, a very serious complication which is known to be frequently fatal However, the over-all mortality for these patients, when treated by intramuscular injections of BAL, was only 22 per cent In 15 of *these patients, the symptoms were described as mild, and* all the patients recovered In 40, the symptoms were described as serious; many of them had convulsions or were in coma Of these 40 patients, 24, or 60 per cent, recovered An analysis of the *results obtained in* the seriously ill patients indicated that BAL must be administered early and in large doses in order to save life The importance of

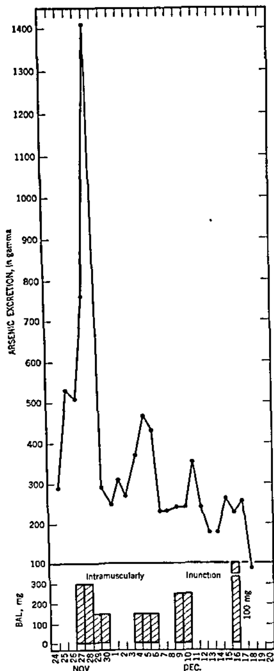


Fig 4. Acute generalized dermatitis, with a temperature of 101 F, in a 19 year old white girl, following administration of 3 6 Gm of neoarsphenamine, Sept 25 to Nov. 11, 1943 Graph shows effect of intramuscular injections of BAL on urinary excretion of arsenic There was rapid improvement after the first course of therapy, followed by relapses which required further treatments

the Canadian workers (26), all of whom reported that administration of BAL increased the urinary excretion of arsenic in animals given toxic doses of arsenical drugs. On the other hand, studies of the urinary excretion of arsenic in man after treatment with BAL

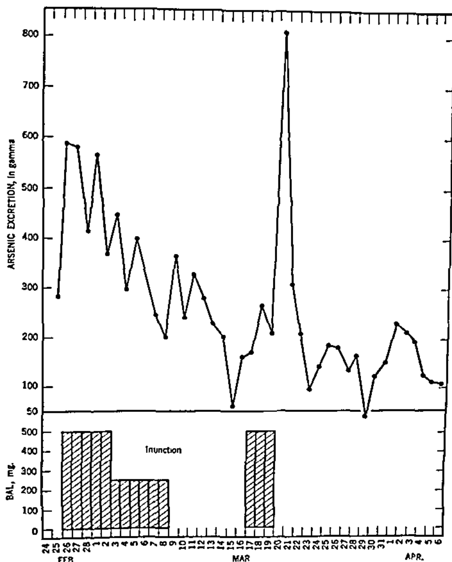


Fig 3 Generalized exfoliative arsenical dermatitis in a 19 year old Negro girl following administration of 3 Gm of neoarsphenamine, Jan 6 to Feb 10, 1943 Graph shows effect of inunctions of BAL in ointment on urinary excretion of arsenic There was symptomatic improvement after the first course of therapy; a relapse required a second course, and this was followed by recovery

urinary arsenic, to detect minimal increments liberated from cells by small amounts of BAL, whereas intensive BAL therapy in patients whose tissues contain considerable arsenic might extract quantities sufficient in amount to produce readily measurable increases in urinary excretion. Because arsenic can be found in the skin of these patients (65), and the visible lesion is in this organ, the patients suffering from dermatitis are particularly good subjects for the study of this problem.

The report by Brown and Hastings (70) that there is increased excretion of arsenic in the urine of patients suffering from alcoholic polyneuritis, as compared to a control group of patients including those suffering from acute infective polyneuritis and from alcoholism without neuritis, has led to the suggestion that arsenic may account, in part at least, for the symptoms in alcoholic neuritis These findings have led them to suggest that BAL might be employed in the treatment of this condition

Mercury Poisoning

The promising results obtained with BAL in the treatment of arsenic poisoning led very shortly to experiments and clinical trials of its use in other forms of metallic poisoning. It has been shown by Barron and associates (33) that BAL combines with a number of heavy metals including lead, cadmium, bismuth, and mercury, forming compounds that are mostly insoluble It has further been established (42,43) that BAL is an inhibitor of enzymes that have as an essential component a metal with which BAL forms complexes.

The application of this knowledge to the experimental therapy of mercury poisoning in animals was first carried out by Gilman and co-workers (31) and a summary of their work was given by Gilman in an earlier publication (71). They employed three preparations: BAL, 1-thiosorbitol; and BAL glucoside All three of these chemicals formed complexes with mercuric chloride *in vitro,* which, however, were toxic in different degrees for animals The complex $Hg(BAL)_2$ proved to be as toxic as $HgCl_2$, and the same was found to be true for $Hg(thiosorbitol)_2$ However, the compound of mercury with one mole of BAL glucoside was significantly less toxic than a molar equivalent amount of $HgCl_2$; and $Hg(BAL\ glucoside)_2$ was only one-eighth as toxic as $HgCl_2$ These results are analogous to

have not yielded altogether consistent results Wexler and associates (34) have studied this problem in normal subjects and in those exposed to diphenylcyanoarsine smoke. They record figures that show a very slight and irregular increase in arsenic excretion in normal men within a period of 6 hours following the single intramuscular injection of 3.5 mg. of BAL; but they found a more definite and consistent increase after an equivalent dose of BAL injected intramuscularly in 11 of 12 men exposed for 6 minutes to a low concentration of diphenylcyanoarsine smoke.

It seems likely, however, from the observations of Luetscher, Eagle, and Longcope (69) that the urinary excretion of arsenic after treatment by BAL may be greatly enhanced by the previous storage of considerable quantities of arsenic in the body; for they found pronounced increases in the arsenical content of 24 hour specimens of urine of 8 patients with arsenical dermatitis who were treated vigorously with inunctions or by intramuscular injections of BAL. Figures 3 and 4 show graphically the changes in arsenic excretion in 2 of these patients, and the relation of the critical increases in urinary arsenic to the administration of BAL. In 4 of these patients there were relapses of the dermatitis, and each responded to a second course of BAL with an increased excretion of arsenic A total of 14 courses of treatment by BAL were given to these 8 patients, with an increased arsenic excretion in the urine during 13 of the 14 courses

Quite different results were obtained in patients with so-called arsphenamine jaundice, for out of 6 such patients only 3 showed any increase in arsenic excretion after treatment with BAL, and in these the increases were very slight These findings accord with those mentioned by Carleton and associates (65) On the other hand, the figures given by Storey, Levvy, and Chance in an appendix to the study of Carleton *et al.* (65), for the urinary excretion of arsenic in 16 cases of arsenical intoxication treated by BAL showed an insignificant or no increase of urinary arsenic either during or following treatment by BAL This is not in accord with the results obtained by Eagle and Magnuson (35), or by Luetscher, Eagle, and Longcope (69).

One explanation for these discrepancies may be found in a variation of results arising from the differences in the dose of BAL employed It might be difficult, in view of the usual variations of

urinary arsenic, to detect minimal increments liberated from cells by small amounts of BAL, whereas intensive BAL therapy in patients whose tissues contain considerable arsenic might extract quantities sufficient in amount to produce readily measurable increases in urinary excretion. Because arsenic can be found in the skin of these patients (65), and the visible lesion is in this organ, the patients suffering from dermatitis are particularly good subjects for the study of this problem

The report by Brown and Hastings (70) that there is increased excretion of arsenic in the urine of patients suffering from alcoholic polyneuritis, as compared to a control group of patients including those suffering from acute infective polyneuritis and from alcoholism without neuritis, has led to the suggestion that arsenic may account, in part at least, for the symptoms in alcoholic neuritis. These findings have led them to suggest that BAL might be employed in the treatment of this condition.

Mercury Poisoning

The promising results obtained with BAL in the treatment of arsenic poisoning led very shortly to experiments and clinical trials of its use in other forms of metallic poisoning. It has been shown by Barron and associates (33) that BAL combines with a number of heavy metals including lead, cadmium, bismuth, and mercury, forming compounds that are mostly insoluble. It has further been established (42,43) that BAL is an inhibitor of enzymes that have as an essential component a metal with which BAL forms complexes.

The application of this knowledge to the experimental therapy of mercury poisoning in animals was first carried out by Gilman and co-workers (31) and a summary of their work was given by Gilman in an earlier publication (71) They employed three preparations: BAL; 1-thiosorbitol; and BAL glucoside All three of these chemicals formed complexes with mercuric chloride *in vitro*, which, however, were toxic in different degrees for animals. The complex $Hg(BAL)_2$ proved to be as toxic as $HgCl_2$, and the same was found to be true for $Hg(thiosorbitol)_2$ However, the compound of mercury with one mole of BAL glucoside was significantly less toxic than a molar equivalent amount of $HgCl_2$; and $Hg(BAL\ glucoside)_2$ was only one-eighth as toxic as $HgCl_2$ These results are analogous to

those with arsenic and cadmium. The *toxicity of the BAL complex* is due to the release of the poisonous metal by dissociation of the complex and oxidation of BAL: an excess of BAL renders it harmless.

In spite of the toxicity of the preformed complexes, both BAL and BAL glucoside proved to be efficient antidotes to acute mercury poisoning in rabbits and dogs. It had previously been shown by Danielli and associates (57) that BAL glucoside, or "BAL intrav." as they termed it, was much less toxic for animals than BAL. It was found to penetrate very slowly into cells so that it acted very largely in the intracellular fluids. BAL glucoside can be administered intravenously.

Gilman and his associates found (31) that rabbits given lethal doses of mercuric chloride intravenously could be saved when injections of any one of the antidotes (BAL, 1-thiosorbitol, or BAL glucoside) were started five minutes later, and given in three doses of 0.1 millimole per kilogram of body weight. The first dose was administered intravenously, followed by the second and third at intervals of 3 hours. By decreasing the dose of the antidotes, BAL glucoside was found to be the most effective, and 1-thiosorbitol the least effective When the interval between the injection of mercuric chloride and the first treatment by BAL was prolonged to 30 minutes, only 50 to 60 per cent of the rabbits could be saved, but even when 1 hour had elapsed an occasional rabbit still survived

Injury to the kidney is one of the serious results of bichloride poisoning; the control rabbits that lived for 24 hours or longer invariably showed an increasing elevation of the urea nitrogen in the blood. Particular stress is laid on the fact that in rabbits treated early with sufficient amounts of BAL or BAL glucoside the evidence of renal insufficiency was completely obviated.

In experiments on dogs, the protective effect of BAL and BAL glucoside against mercury poisoning was even more striking than in rabbits. Dogs given lethal doses of $HgCl_2$ intravenously were completely protected from the renal effects of the metal by 3 equal doses of BAL totaling 0.15 mM per kilogram when treatment was delayed for 30 minutes. Striking protection was still afforded both by BAL and BAL glucoside when treatment was delayed for as long as 2 hours. When mercury bichloride was administered to dogs by mouth and vomiting was prevented, the lethal effects of the

poison could be largely prevented even when treatment with BAL or BAL glucoside was not started until a lapse of 3 or 5 hours. Thus, only 4 of 11 dogs died when the first of 3 doses of BAL or BAL glucoside was administered 5 hours after the oral dose of mercury; of the 9 animals surviving more than 48 hours, only 2 showed evidences of renal insufficiency. Confirmation of this work has been reported by Braun, Lusky, and Calvery (50), who were able to obtain similar results in rabbits.

Acute intoxication of animals by organic materials can also be combated by BAL. Long and Farah (51) have shown that the acute toxicity of salyrgan, the mercurial diuretic, can be readily counteracted in mice, cats, and dogs by sulfhydryl containing compounds such as glutathione, cysteine, and 2,3-dimercapto-1-propanol (BAL). The most effective of these was BAL, which, in equimolar doses, was about 5 times as active as cysteine or glutathione.

With knowledge of some of this experimental work upon the subject, we (72) undertook in 1944 to treat patients with mercurial poisoning by intramuscular injections of 10 per cent solutions of BAL in benzyl benzoate and peanut oil. Since the publication of a paper describing the details relating to the treatment of 23 patients, 38 additional cases have been studied and a final analysis can now be made of the entire series of 61 patients

With the cooperation of the police department and the hospitals in Baltimore, all known cases of bichloride poisoning were brought at the earliest possible moment to a special ward in the Johns Hopkins Hospital Some of these patients were given emergency treatment before admission to the Johns Hopkins Hospital by lavage of the stomach with sodium formaldehyde sulfoxylate, though a few received only milk and eggs by stomach tube On admission to the Johns Hopkins Hospital, lavage of the stomach with 5 or 10 per cent sodium formaldehyde sulfoxylate was employed as a routine procedure At the same time, 300 mg. of BAL in a 10 per cent solution in benzyl benzoate and peanut oil was injected intramuscularly; 1 to 2 hours after this initial dose, the patients were given 150 mg of BAL and this was usually followed within 4 to 6 hours by another dose of 150 mg. In patients who had swallowed large amounts of bichloride, a third dose of 150 mg. was often given within the first 12 hours after admission, so that many patients received 600 to 750 mg. of BAL during the first 12 hours after

admission. During the succeeding 24 hours, 2 to 3 doses of 150 mg. of BAL were injected. Injections of BAL were continued, usually in diminishing amounts according to the severity of symptoms, for the subsequent 2 to 4 days, though in a few instances of especially severe poisoning, BAL was continued in doses of 150 to 300 mg for 5 to 7 days. Thus, in 18 cases the total amount of BAL administered during this period amounted to from 1.5 Gm. to 3 Gm of BAL.

Despite the comparatively large initial doses of BAL, which often exceeded the recommended amount of 2 to 3 mg. per kilogram of

TABLE I

Results of Treatment with BAL in Sixty-One Patients with Bichloride of Mercury Poisoning

Amount bichloride of *mercury taken*, Gm.	*Number of* patients	*Number* recovered	*Number* died
0 5	20	20	0
1	18	17	1
1 5	12	12	0
2 and over	11	10	1
Totals	*61*	*59*	*2*

body weight, and despite the rather large total amounts injected, untoward symptoms referable to BAL were rare. An occasional patient complained of tingling of the tongue and lips, or of the extremities, and a few had transient abdominal pain. One patient experienced flushing of the face, fullness in the head, sweating, shooting pains in the arms and legs, and burning in the epigastrium after a dose of 300 mg of BAL, and two women were observed to have transient cardiac irregularities due to extrasystoles following single doses of 150 mg of BAL. A great many patients showed a moderate elevation of blood pressure within the first 24 to 48 hours after admission, but it is questionable whether this was due to BAL.

The severity of symptoms from mercurial poisoning in these 61 patients depended largely upon the amounts of bichloride which they were supposed to have swallowed. This varied from a single tablet of 0.5 Gm. to an estimated amount of 20 Gm. of powdered bichloride in water (Table I). Of the 20 patients who swallowed not more than 0.5 Gm. none were seriously ill on admission, and all

recovered completely within 2 to 7 days; 11 of these patients showed a transient albuminuria, and in 10 cases there was a leukocytosis varying from 12,000 to 20,000. In 8 of 10 cases, the reaction to tests for mercury in the stools was positive.

Of the 18 patients who had swallowed at least 1 Gm of bichloride, a number were seriously ill with nausea, vomiting, and abdominal pain; 10 of them had bloody diarrhea, 14 had leukocyte counts varying from 12,000 to 24,000, many showed hemoconcentration, and in 5 the hematocrit was above 50. All but 2 had well-marked albuminuria and 5 of the 6 patients in whom tests were made for mercury in the urine gave positive results. One patient, admitted in profound shock 13 hours after she had swallowed bichloride and had slashed her wrists in a suicidal attempt, died. The remaining 17 patients recovered completely within 2 to 8 days after admission.

Of the 23 patients who had swallowed from 1 5 to about 20 Gm. of bichloride, all were seriously poisoned There was severe abdominal pain and tenderness; vomiting was persistent, and in almost all the vomitus contained blood, often in large amounts. Bloody diarrhea was constant. In several, the tongue was slate-colored. In a few, there were superficial ulcers on the tongue, gums, or pharynx. Two or three of the patients were semistuporous, in part perhaps due to alcohol. Several were in shock, and required blood transfusions in addition to the other forms of therapy employed. Hemoconcentration was often extreme, the total plasma proteins in 9 of the 12 cases in which they were determined varied from 8 to 10.19 Gm The plasma carbon dioxide combining power was frequently reduced, reaching 14 to 17 mEq in 4 patients. A marked leukocytosis was almost invariable, reaching 20,000 to 34,000 in 6 patients Mercury was present, often in large amounts, in the stools of all the 13 patients in whom tests were made, and was found in the urine of 7 patients All patients showed marked albuminuria and cylindruria It was somewhat surprising to find that the stools of several of these patients gave persistently positive tests for mercury for many hours or even days after admission In 5 patients, the tests were negative after approximately the first 12 hours, in 6, mercury was demonstrated during the next 24 hours; in 1, through 48 hours, in 1, continuously for 5 days; and in 1 patient, continuously for 8 days, and then again after an exacerbation of symptoms on the fifteenth day

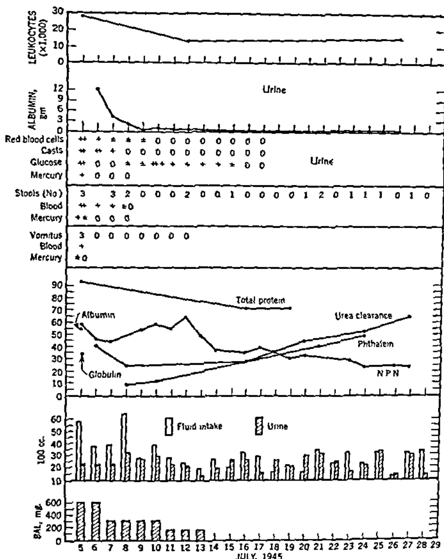


Fig 5 Chart of a 16 year old Negro girl admitted to Johns Hopkins Hospital on July 5, 1945 At 10 p.m on July 4 (19 hours before admission), she had taken 3 tablets (1.5 Gm) of bichloride of mercury in water; 15 minutes later she drank 3 quarts of milk and vomited Milk and eggs were administered 1 to 2 hours later. The vomiting continued, and by 4 a.m was bloody Abdominal pain continued, and she became drowsy On admission, the stomach was lavaged with 5 per cent sodium formaldehyde sulfoxylate, physiologic saline and 5 per cent glucose solution was administered intravenously, and intramuscular injections of BAL were started. She passed 2 bloody stools, was stuporous, and had pitting edema Recovery was complete

A 55 year old woman who had swallowed at least 2 Gm of bichloride, and was not admitted until 6 hours later, died. The remaining 22 patients recovered, 12 within 3 to 7 days. A young colored woman, admitted 19 hours after taking 1.5 Gm. of bichloride (Fig. 5), and in whom on admission there was evidence of renal insufficiency with edema, did not recover completely for 22 days; and 1 man admitted 1 hour after he had swallowed 3 Gm. of bichloride, in whom bronchopneumonia due to pneumococcus type II developed, together with renal insufficiency, did not recover for 33 days (Fig. 6A). The latter case is also instructive from another viewpoint. As can be seen from the chart, a reduction in urinary output and an increase in the nonprotein nitrogen of the blood occurred when the doses of BAL were omitted or decreased. Concurrently mercury was still present in the stools. Reinstitution of injections of BAL in amounts of 300 mg a day was coincidental with an improvement in the patient's condition and was accompanied by a decrease in the nonprotein nitrogen of the blood and a disappearance of mercury from the stools. That mercury was not completely eliminated from the body even at this time is evidenced by the single reappearance of mercury in one specimen of stool 7 days later. The situation in this patient is reminiscent of the frequent relapses that have been observed in arsenical dermatitis during treatment with BAL.

With the exception of the 2 fatal cases and the 2 patients with prolonged illnesses, the chief features of which are shown in Figures 5 and 6A–B, all the patients who had swallowed 1 Gm. or more of bichloride of mercury recovered with surprising and unexpected rapidity In this group of 41 patients, there were many who were admitted to the hospital in desperate condition, and several were in collapse Vomiting of blood was common, and profuse diarrhea with hemorrhagic stools was present in the majority. There was noticeable improvement within 24 hours after the institution of BAL therapy, and within 48 hours vomiting had ceased and the diarrhea was controlled. By this time, appetite had returned, the superficial ulcerative lesions in the mouth, when present, were healing, and a number of patients desired to get up and go home. None of these 41 patients who had swallowed 1 Gm. or more of $HgCl_2$ developed persistent diarrhea; in none was there progressive loss of weight; none showed a complicating anemia; and in none were there chronic

ulcerating lesions of the buccal mucosa. The restitution to a comparatively healthy state was therefore rapid, and as far as could be determined, complete.

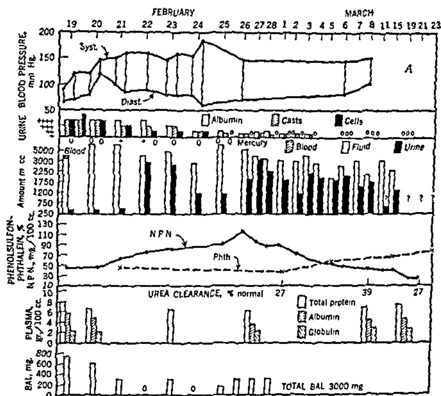


Fig. 6A Chart of a 42 year old man admitted to Johns Hopkins Hospital on February 19, 1916, at 2 50 p.m Less than an hour earlier he had swallowed 6 tablets (3 Gm) of bichloride of mercury, followed by a bottle of Coca Cola, 30 minutes later he had vomited Intramuscular injections of BAL were started 1 hour after he had swallowed the poison. By that time he was in shock, vomiting blood, and passing bloody stools Recovery was complete.

The chart shows the following the course of blood pressure, the results of urinalysis; results of qualitative tests for mercury, nonprotein nitrogen of the blood; phenolsulfonphthalein excretions; urea clearance; plasma proteins, and amounts of BAL administered

The comparatively small quantities of urine voided by these patients during the first few hours after admission contained vary-

ing amounts of albumin, casts, cells, and sometimes red blood corpuscles. It was rare, however, to find an elevation of the nonprotein nitrogen of the blood. In only 3 of the 39 surving patients did it reach figures above 42 mg. per hundred cubic centimeters Within

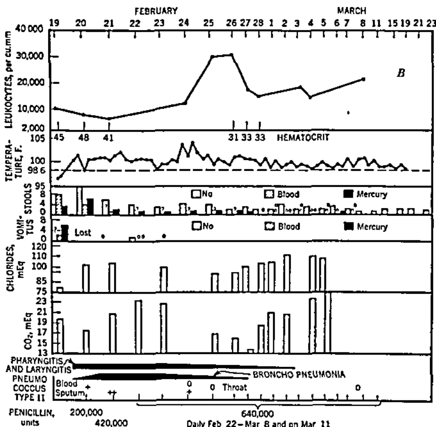


Fig 6B Chart of same patient as in Fig 6A· leukocyte curve, hematocrit levels, temperature, analyses of stools and vomitus, with qualitative tests for mercury, chlorides and carbon dioxide combining power of the blood, and course of intercurrent infections, with amounts of penicillin administered.

48 hours after treatment with BAL was started the patients voided large quantities of urine which soon became free of albumin and cellular elements. The results of treatment in these patients were comparable, therefore, to those obtained experimentally in rabbits

and dogs; for the animals that survived lethal doses of bichloride of mercury after prompt administration of BAL rarely showed a subsequent rise in the urea nitrogen of the blood.

In other respects, too, the results in man are analogous to those in experimental animals, for an analysis of the outcome in these 61 patients brings out the great importance of early treatment with BAL. Of the entire 61 patients, only 2 died; and it is to be noted that in these 2 fatal cases treatment was not started until 13 hours and 6 hours, respectively, after the poison had been swallowed

It is difficult to obtain accurate information concerning the fatal oral dose of bichloride of mercury, but it is usually stated (73,74) that a dose of 0 5 Gm is rarely if ever fatal; that when 1 Gm. is swallowed and vomiting does not occur within 10 minutes the prognosis is poor; and that 1 5 Gm. often results in death.

In order to obtain some information concerning the fate of cases of mercury poisoning in whom treatment by the older conventional methods was started within 4 hours after the patients had swallowed the poison, 2 groups of patients were compared. The first group was composed of 86 patients who had swallowed 1 Gm. or more of bichloride of mercury and who were treated within 4 hours by the conventional methods at the Johns Hopkins Hospital; the second group comprised the 38 patients who had swallowed 1 Gm. or more of bichloride of mercury and in whom treatment with BAL had been started within 4 hours. The results are.

	Number of cases	Number of deaths
Control cases	86	27
Patients treated with BAL	38	0

It appears, therefore, from the available data, that the efficacy of BAL in the treatment of oral mercurial poisoning depends not so much upon the amount of bichloride that has been swallowed as upon the interval of time between the ingestion of the poisonous chemical and the first intramuscular injections of sufficiently large doses of BAL The results obtained in this series of patients indicate that BAL can act as an effective antidote to mercurial intoxication in man, for it appears that BAL, in sufficient amounts, can combine with very large quantities of mercury in the body, forming a complex that is innocuous and that may be excreted rapidly

through the kidneys, without producing any demonstrable injury to this organ.

It may happen occasionally that a patient who has swallowed an amount of mercury bichloride which is usually fatal and who is treated by adequate dosages of BAL only after a number of hours may recover, as occurred in 1 of the 3 reported patients in this category. The chances, however, are that injury to the kidneys will be so far advanced that the changes are irreversible and the patient will die.

Poisoning by Other Metals

The generalization that heavy metals possess a common mechanism of injurious action and that this can be antagonized by dithiols has stimulated experiments in search of further support of this conception. It has been found in the course of these investigations, however, that the complexes that BAL and other thiols form *in vitro* with metals may themselves be as toxic as the original metal. The chemical processes involved in the combinations of cadmium with BAL, BAL glucoside, and the monothiols *l*-thiosorbitol and 1-thioxylitol have been described and discussed by Gilman and co-workers (32). They have pointed out that these complexes may be dissociated *in vivo*, by which method the toxic metal could be released and thus produce an injurious effect, often of a totally unexpected type. When prophylactic injections of BAL were given to rabbits that were later poisoned by lethal or sublethal doses of cadmium intravenously, the animals were in large part protected against the acute toxic symptoms, which otherwise were usually fatal within 24 hours; nevertheless, these rabbits died after several days with acute renal insufficiency due to extensive lesions in the kidneys The explanation offered for this result was that the complex formed *in vivo* between BAL and cadmium was relatively stable, but still susceptible to intracellular oxidation In the presence of an excess of BAL, the metal was directed toward the kidney for excretion, where, through glomerular filtration and tubular reabsorption, intracellular oxidation and dissociation occurred. The free cadmium was then capable of acting upon the renal epithelial cells and of producing in them a lesion such as rarely if ever occurred in the control animals dying of cadmium poisoning alone.

BAL glucoside gave somewhat better protection than BAL against the acute symptoms, and the animals surviving the acute intoxication were less likely to show evidence of renal injury.

These experiments are of great interest, but since the major danger of cadmium poisoning for man comes from the inhalation of smokes and toxic gases, particularly in certain forms of industry, the experiments of Tobias and associates (53) are of practical importance They made an extensive study of the pathologic effects in mice and dogs of cadmium poisoning induced by inhalation of cadmium chloride dust The essential lesion is an acute pulmonary edema followed by bronchopneumonia or pneumonitis The animals may die within 48 hours of acute pulmonary edema, or after a week or longer from pneumonitis

In mice, intramuscular injections of BAL started 1 to 3 hours after exposure to cadmium dust and repeated every 2 hours for 6 to 8 hours proved effective in saving the life of these animals In one experiment, the result was a mortality of 93 per cent for untreated mice and 7 per cent for the mice treated with BAL As in other forms of metallic poisoning, the time elapsing between intoxication and treatment was critical When the first dose of BAL was withheld until 3 to 6 hours after intoxication, the results were dubious; and when 6 to 12 hours had elapsed, the injections of BAL seemed actually to hasten death

As a prophylactic, BAL proved to be useless or actually detrimental, which is an observation comparable to that obtained by Gilman and associates (32) in rabbits It appears, further, that when cadmium was injected intravenously into dogs and was followed by intravenous injections of BAL intense renal changes resulted, thus conforming again to the results obtained in rabbits (32) Poisoning by inhalation of cadmium was not as readily controlled by BAL in dogs as in rabbits The survival time was prolonged and the mortality reduced, but favorable effects of treatment were not as striking as they were in mice

Results somewhat analogous to these have been reported by Braun, Lusky, and Calvery (50) for lead, thallium, and selenium They found that BAL was ineffective in the treatment of rabbits poisoned by the salts of these metals and in the case of lead and selenium BAL actually had an additive effect However, they offer no explanation for these results On the other hand, they found that

BAL proved to be an effective antidote in acute poisoning of rabbits caused by the administration of the salts of antimony, bismuth, strontium, and nickel. In their short series of experiments, they found that treatment by BAL increased the tolerance of rabbits to lethal doses of these metals by at least 50 per cent.

Gammill, Southam and Van Dyke (45) have reported experiments to show that BAL is an effective antidote against poisoning by tartar emetic (antimony potassium tartrate) injected intraperitoneally or intramuscularly into rats. In a small number of experiments, the curative property of BAL seemed to be quite as noticeable in rats poisoned with tartar emetic as in rats poisoned by mercury bichloride. On the other hand, BAL appeared to enhance the toxicity and increase the mortality rate from fuadin and neostam or neostibosan, which is contrary to the findings of Braun, Lusky, and Calvery (50) who recorded equally as satisfactory therapeutic results from BAL in rabbits poisoned by fuadin and neostam as by antimony tartrate. Eagle and co-workers (52) found that large and repeated doses of BAL were necessary to save 50 per cent of the animals given a lethal dose of antimony in the form of fuadin, anthiomaline, tartar emetic, or *p*-methylphenylstibonic acid. They employed BAL in doses of 10 to 15 mg per kilogram every 4 hours for 4 doses on the day of poisoning, followed by 1 or 2 injections for the next 2 to 4 days. Even more intensive and prolonged therapy is suggested as a possible method of improving the results.

These authors (52) demonstrated a striking increase in the urinary excretion of antimony when animals poisoned with fuadin were given BAL. Definite rises in antimony excretion followed BAL treatment of animals which had received toxic doses of anthiomaline and tartar emetic. BAL had little effect, however, on the excretion of antimony after *p*-methylphenylstibonic acid poisoning.

One series of observations by Olcott and Riker (54) on experimental argyria in rats indicates that BAL is incapable of mobilizing silver deposited in the tissues. This leads them to conclude that BAL is not indicated as a treatment for argyria in man.

BAL has been used with apparent success in the treatment of acute gold poisoning in man It is well known that gold salts treatment of patients suffering from rheumatoid arthritis may result in one of a number of toxic reactions. Among the commoner manifestations of this intoxication are dermatitis and glossitis and among

some of the more unusual ones are nephrosis, purpura, and granulocytopenia. Cohen, Goldman, and Dubbs (75) report 5 cases of acute gold poisoning treated with BAL; Ragan and Boots (76) report 5 cases; and Lockie, Norcross, and George (77) record 2 cases. Among these instances of gold intoxication there were 9 cases of dermatitis (75,76), one of stomatitis (75), and one each of thrombopenic purpura and granulocytopenia (77). In 8 of the cases of dermatitis, in which the rash had lasted from a few days to less than 2 months, the results of treatment by BAL were considered to be satisfactory and at times dramatic (75,76). In 1 patient who had suffered from a dermatitis for at least 3 months, the rash was resistant to treatment by BAL (76). The response of the patient with gold stomatitis to BAL therapy was considered to be remarkably good (75). The recovery of the patients suffering from thrombopenic purpura and from granulocytopenia was described as "spectacular" (77). An increased excretion of gold in the urine accompanied the administration of BAL in 5 of the patients with dermatitis (76). This was quite as marked in the patient who did not respond favorably as it was in those who showed satisfactory improvement of the dermatitis. In 4 of these 5 patients, the symptoms of rheumatoid arthritis became aggravated within a month after treatment by BAL; this suggested that the elimination of gold from the tissues through the action of BAL had rendered the patient susceptible to a relapse of the rheumatoid arthritis (76).

It seems evident that further observations on the effect of BAL should be made on patients suffering from acute poisoning from gold

Reference has been made (50) to the ineffectiveness of BAL in protecting animals against lead poisoning and to the fact that it may actually have an additive effect. More recent experiments have, however, thrown further light on this subject, for Ryder, Cholak, and Kehoe (78) found, after injections of BAL, significant decreases of lead in the blood of lead workers, some of whom had symptoms of poisoning This decrease was evident shortly after the injection of BAL, and reached its maximum in 8 hours A return to previous levels occurred within 24 hours Concomitant with the decrease of lead in the blood, there was an increase in the concentration of lead in the urine Repeated injections of BAL produced less and less effect, did not affect symptoms, and did not shorten the course of the disease. We (79) have observed one patient with subacute

lead poisoning, in whom a temporary increase in the urinary excretion of lead occurred during the administration of BAL.

The problem has been studied experimentally in rabbits by Germuth and Eagle (80,81). They found that 5 consecutive daily subcutaneous injections of lead acetate caused the death of control rabbits within 3 to 40 days after the last injection. When the salt was given intravenously in much smaller amounts, it produced an acute poisoning from which the animals died in an average of 3 days. The intramuscular administration of BAL failed to protect animals poisoned with lead acetate by either route of administration and some rabbits died even faster than the corresponding untreated controls. These results are in accord, therefore, with those reported by Braun, Lusky, and Calvery (50). However, the administration of BAL caused a marked increase in the urinary secretion of lead, which lasted for 2 hours after a single injection of BAL. When repeated doses of BAL were employed, a progressive decrease in the amount of lead in the urine occurred after each injection. It was found that a lead–Bal complex, formed by adding a solution of lead acetate to an aqueous solution of BAL *in vitro,* was quite as toxic as the lead acetate itself They conclude:

"The failure of BAL to protect these animals may be in part due to the fact that it mobilizes only a small fraction of the total body store of lead, and in part to the fact that the lead–BAL complex proved almost as toxic as the lead salt itself on intravenous injection."

These observations and experiments indicate that much more information will have to be obtained about the dangers and possible benefits arising from the use of BAL for lead poisoning before BAL can be used therapeutically.

In surveying all the information that is available concerning the biochemistry of BAL, its reactions with metallic poisons, and its therapeutic effects in man, one is impressed particularly with the fundamental importance of the experimental investigations that have been made upon the subject. They have thrown further light upon the manner in which metallic poisons combine with intracellular enzymes to destroy vital processes in cells, and have elucidated the mechanism by which BAL blocks this injurious action It is very likely that these discoveries will have wide-reaching significance, and will bear upon many fields of research

The practical application of this information to the therapy of some metallic poisons has shown that BAL is very effective in the treatment of mercury poisoning, and of some forms of arsenical intoxication It may be useful in combatting the injurious effects of gold salts, and possibly of some other metals It seems doubtful whether BAL can be employed to advantage in lead poisoning, or in most instances of cadmium poisoning

Remark

Since the writing of this chapter in the late summer of 1947 a number of papers have appeared which could not be incorporated into the review without complete resetting of the type. It was possible, however, to add in proof the above few paragraphs referring to recent experimental work on lead poisoning.

References

1. Ehrlich, P., and Hata, S.: The Experimental Chemotherapy of Spirilloses. New York, Rebman, 1911.
2. Voegtlin, C., and Thompson, J. W.: Quantitative studies in chemotherapy. VI. Rate of excretion of arsenicals, a factor governing toxicity and parasiticidal action. J. Pharmacol. & Exper. Therap. *20*, 85, 1922.

3a. Thuret, J.: La fixation "in vitro" des composés arsénicaux par les globules rouges du sang. J. de pharm. et chim. *28*, 22, 1938.

3b. Thuret, J.: La fixation "in vivo" des composés arsénicaux par les globules rouges du sang. J. de pharm. et chim. *28*, 60, 1938.

4. Hogan, R. B., and Eagle, H.: The pharmacologic basis for the widely varying toxicity of arsenicals. J. Pharmacol. & Exper. Therap. *80*, 93, 1944.
5. Heffter, A.: Die reduzierenden Bestandteile der Zellen. Med.-naturw. Arch. *1*, 81, 1907.
6. Ehrlich, P.: Über den jetzigen Stand der Chemotherapie. Ber. d. deutsch. chem. Gesellsch. *1*, 17, 1909.
7. Walker, E.: Chemical constitution and toxicity. Biochem. J. *22*, 292, 1928.
8. Rosenthal, S. M.: Action of arsenic upon the fixed sulfhydryl groups of proteins. Pub. Health Rep. *47*, 241, 1932.
9. Voegtlin, C., Dyer, H. A., and Leonard, C. S.: On the mechanism of the action of arsenic upon protoplasm. Pub. Health Rep. *38*, 1882, 1923.
10. Szent-Gyorgyi, A.: The action of arsenite on tissue respiration. Biochem. J. *24*, 1723, 1930.
11. Barron, E. S. G., and Singer, T. P.: Enzyme systems containing active sulfhydryl groups. The role of glutathione. Science *97*, 356, 1943.
12. Peters, R. A., Sinclair, H. M., and Thompson, R. H. S.: An analysis of the inhibition of pyruvate oxidation by arsenicals in relation to the enzyme theory of vesication. Biochem. J. *40*, 516, 1946.
13. Thompson, R. H. S.: The effect of arsenical vesicants on the respiration of the skin. Biochem. J. *40*, 525, 1946.
14. Hellerman, L.: Reversible inactivations of certain hydrolytic enzymes. Physiol. Rev. *17*, 454, 1937.
15. Hellerman, L., Chinard, F. P., and Deitz, V. R.: Proteins sulfhydryl groups and the reversible inactivation of the enzyme urease. J. Biol. Chem. *147*, 443, 1943.
16. Peters, R. A., and Wakelin, R. W.: Observations upon the relation

between sulfhydryl groups and pyruvate oxidation in brain tissue Biochem J. *40,* 513, 1946

17 Labes, R.: Über die pharmakologische Bedeutung der chemischen Reaktionen zwischen arseniger Saure und Thiolverbindungen Arch f. exper path u. Pharmakol. *141,* 148, 1929

18 Cohen, A, King, H, and Strangeways, W. I: Trypanocidal action and chemical constitution X. Arylthioarsinites. J. Chem. Soc. p 3043, 1931.

19 Strangeways, W I · The trypanocidal action in vitro of aromatic thioarsinites and neoarsphenamine Ann Trop Med *31,* 387, 1937.

20 Eagle, H. The effect of sulfhydryl compounds on the antispirochetal action of arsenic, bismuth, and mercury compounds in vitro J. Pharmacol & Exper Therap *66,* 436, 1939

21 Stocken, L A, and Thompson, R. H. S. British anti-lewisite 1. Arsenic derivatives of thiol proteins Biochem. J *40,* 529, 1946

22 Whittaker, V P An experimental investigation of the "ring hypothesis of arsenical toxicity Biochem J. *41,* 56, 1947

23 Stocken, L A, and Thompson, R H S British anti-lewisite 2 Dithiol compounds as antidotes for arsenic. Biochem J *40,* 535, 1946

24 Peters, R A, Stocken, L A., and Thompson, R. H S · British anti-lewisite (BAL) Nature *156,* 616, 1945

25 Waters, L. L, and Stock, C: BAL (British anti-lewisite). Science *102,* 601, 1945

26 Young, L Canadian researches on BAL (British anti-lewisite) Science *103,* 439, 1946

27. Barron, E S G, Miller, Z B, Bartlett, G R, Meyer, J, and Singer, T P Reactivation by dithiols of enzymes inhibited by lewisite. Biochem J *41,* 69, 1947

28 Eagle, H, Magnuson, H J, and Fleischman, R The systemic treatment of experimental arsenic poisoning (mapharsen, lewisite, phenyl arsenoxide) with BAL J Clin Investigation *25,* 541, 1946

29 Stocken, L A, and Thompson, R. H S British anti-lewisite 3 Arsenic and thiol excretion in animals after treatment of lewisite burns Biochem. J *40,* 548, 1946

30 Peters, R A, and Stocken, L A Preparation and pharmacological properties of mapharside-BAL compound Biochem J *41,* 53, 1947

31 Gilman, A, Allen, R P, Philips, F S and St John, E · The treatment of acute systemic mercury poisoning in experimental animals with BAL, thiobital, and BAL glucoside, J Clin Investigation *25,* 549, 1946

32 Gilman, A, Philips, F S, Allen, R P, and Koelle, E S.: The treatment of acute cadmium intoxication in rabbits with BAL and other mercaptans J Pharmacol & Exper Therap 87, 85 (Suppl), 1946

33 Barron, E S G, Miller, Z B, and Kalnitsky, G The oxidation of dithiols Biochem J *41,* 62, 1947

34 Wexler, J, Eagle, H, Tatum, H J, Magnuson, H J, and Watson, E S: The effect of BAL on the excretion of arsenic in normal subjects and

Remark

Since the writing of this chapter in the late summer of 1947 a number of papers have appeared which could not be incorporated into the review without complete resetting of the type. It was possible, however, to add in proof the above few paragraphs referring to recent experimental work on lead poisoning

References

1. Ehrlich, P, and Hata, S. The Experimental Chemotherapy of Spirilloses New York, Rebman, 1911
2. Voegtlin, C., and Thompson, J. W · Quantitative studies in chemotherapy. VI. Rate of excretion of arsenicals, a factor governing toxicity and parasiticidal action J. Pharmacol & Exper Therap *20*, 85, 1922.
3a *Thuret, J.: La fixation "in vitro" des composés arsénicaux par les globules* rouges du sang J. de pharm et chim *28*, 22, 1938
3b Thuret, J La fixation "in vivo" des composés arsénicaux par les globules rouges du sang J de pharm et chim *28*, 60, 1938
4 Hogan, R. B, and Eagle, H The pharmacologic basis for the widely varying toxicity of arsenicals J Pharmacol & Exper Therap *80*, 93, 1944
5 Heffter, A: Die reduzierenden Bestandteile der Zellen Med-naturw Arch *1*, 81, 1907.
6. Ehrlich, P. Über den jetztigen Stand der Chemotherapie Ber d deutsch chem Gesellsch *1*, 17, 1909
7 Walker, E · Chemical constitution and toxicity Biochem J *22*, 292, 1928
8 Rosenthal, S M Action of arsenic upon the fixed sulfhydryl groups of proteins Pub Health Rep. *47*, 241, 1932
9 Voegtlin, C, Dyer, H A, and Leonard, C S On the mechanism of the action of arsenic upon protoplasm Pub Health Rep *38*, 1882, 1923
10 Szent-Gyorgyi, A.. The action of arsenite on tissue respiration. Biochem J *24*, 1723, 1930
11 Barron, E S. G., and Singer, T. P.: Enzyme systems containing active sulfhydryl groups. The role of glutathione Science *97*, 356, 1943
12 Peters, R. A, Sinclair, H. M, and Thompson, R H S An analysis of the inhibition of pyruvate oxidation by arsenicals in relation to the enzyme theory of vesication Biochem J *40*, 516, 1946.
13 Thompson, R. H. S: The effect of arsenical vesicants on the respiration of the skin Biochem J *40*, 525, 1946
14. Hellerman, L Reversible inactivations of certain hydrolytic enzymes Physiol Rev *17*, 454, 1937
15 Hellerman, L, *Chinard, F. P, and Deitz, V. R · Proteins sulfhydryl groups* and the reversible inactivation of the enzyme urease. J Biol Chem *147*, 443, 1943
16. Peters, R. A., and Wakelin, R W.. Observations upon the relation

50 Braun, H A, Lusky, L M, and Calvery, H O.: The efficacy of 2,3-dimercaptopropanol (BAL) in the therapy of poisoning by compounds of antimony, bismuth, chromium, mercury, and nickel. J. Pharmacol. & Exper. Therap *87*, 119 (Suppl), 1946

51 Long, W. K., and Farah, A.: The influence of certain sulfhydryl compounds on the toxicity of an organic mercurial diuretic. J. Pharmacol & Exper. Therap *88*, 388, 1946.

52 Eagle, H, Germuth, F. G, Jr, Magnuson, H. J., and Fleischman, R: The protective action of BAL in experimental antimony poisoning J Pharmacol & Exper. Therap. *89*, 196, 1947.

53. Tobias, J. M, Lushbaugh, C. C, Patt, H. M., Postel, S, Swift, M. N., and Gerard, R W.: The pathology and therapy with 2,3-dimercaptopropanol (BAL) of experimental cadmium poisoning J. Pharmacol. & Exper. Therap *87*, 102 (Suppl), 1946

54. Olcott, C. T, and Riker, W. F.: Experimental argyrosis. II Treatment of rats receiving silver with 2,3-dimercaptopropanol (BAL). Science *105*, 67, 1947

55 Chenoweth, M B, Modell, W., and Riker, W F., Jr.: Pharmacology of several dithiols related to 2,3-dimercaptopropanol (BAL). J. Pharmacol. & Exper Therap. *87*, 6 (Suppl), 1946

56 Fitzhugh, O G, Woodard, G, Braun, H A, Lusky, L M, and Calvery, H O: J. Pharmacol & Exper Therap *87*, 23 (Suppl), 1946.

57 Danielli, J F, Danielli, M., Mitchell, P D., Owen, L. N., and Shaw, G: Development of a chemotherapy for systemic arsenical poisoning. Nature, London *157*, 217, 1946.

58 McCance, R A., and Widdowson, E M. Observations on the administration of BAL-Intrav to man Nature, London *157*, 837, 1946

59 McDonald, I W: Effect of BAL-Intrav on excretion of copper by the sheep Nature, London *157*, 837, 1946.

60 Fell, H B, and Allsop, C B: Effects of lewisite and lewisite oxide on living cells in vitro Brit J Exper Path *27*, 305, 1946

61 Fell, H. B, and Allsop, C B Therapeutic effect of British anti-lewisite (BAL) on tissue cultures grown in medium containing lewisite oxide Brit J Exper Path *27*, 310, 1946

62 Mann, I, Pirie, A, and Pullinger, B D Treatment of lewisite lesions with BAL Am J Ophth *30*, 421, 1947

63 Hughes, W F Clinical uses of 2,3-dimercaptopropanol (BAL). IX. The treatment of lewisite burns of the eye with BAL J Clin. Investigation *25*, 541, 1946

64 Sulzberger, M B, Baer, R L, and Kanof, A Skin sensitization to BAL J Clin Investigation *25*, 488, 1946

65 Carleton, A B, Peters, R A, Stocken, L A, Thompson, R H. S, and Williams, D I The treatment of complications of arsenotherapy with BAL J Clin Investigation *25*, 497, 1946

65a Carleton, A B, Peters, R. A, and Thompson, R H S: The treatment of arsenical dermatitis with dimercaptopropanol (BAL) Quart J. Med *17*, 49, 1948

after minimal exposure to arsenical smoke J. Clin. Investigation *25*, 467, 1946.

35 Eagle, H, and Magnuson, H. J.: The systemic treatment of 227 cases of arsenic poisoning (encephalitis, dermatitis, blood dyscrasias, jaundice, fever) with 2,3-dimercaptopropanol (BAL). *Am J. Syphilis 30, 420, 1946*

36. Sulzberger, M. B., Baer, R. L, and Kanof, A · Studies on the toxicity of BAL on percutaneous and parenteral administration J. Clin Investigation *25*, 474, 1946

37. Modell, W, Gold, H, and Cattell, M: Pharmacologic observations on BAL by intramuscular injection in man J Clin Investigation *25*, 480, 1946

38. Durlacher, S H, Bunting, H, Harrison, H. E, Ordway, N K, and Albrink, W S: The toxicological action of 2,3-dimercaptopropanol (BAL) J. Pharmacol. & Exper Therap. *87*, 28 (Suppl), 1946

39. Modell, W, Chenoweth, M B, and Krop, S · Toxicity of 2,3-dimercaptopropanol (BAL) *in cats J Pharmacol. & Exper Therap 87, 33 (Suppl)*, 1946

40 Chenoweth, M. B The cardiovascular actions of 2,3-dimercaptopropanol (BAL) J. Pharmacol. & Exper Therap *87*, 41 (Suppl), 1946.

41. Hitchcock, P.. Effects of dithiols and other enzyme inhibitors on blood vessels J. Pharmacol & Exper Therap *87*, 55 (Suppl), 1946

42 Webb, E. C., and Van Heyningen, R The action of British anti-lewisite (BAL) on enzyme systems Biochem J *41*, 74, 1947

43 Barron, E S G, Miller, Z B, and Meyer, J · The effect of 2,3-dimercaptopropanol on the activity of enzymes and on the metabolism of tissues. Biochem. J *41*, 78, 1947

44 *Stocken, L A, Thompson, R. H S, and Whittaker, V P · British anti*-lewisite 4 Antidotal effects against therapeutic arsenicals Biochem J *41*, 47, 1947.

45 Gammill, J F, Southam, C M, and Van Dyke, H B Effect of 2,3-dimercaptopropanol (BAL) on acute poisoning by tervalent and quinquevalent antimonial drugs Proc Soc Exper Biol & Med *64*, 13, 1947

46 Harrison, H E, Ordway, N K, Durlacher, S H, Albrink, W S, and Bunting, H · Poisoning from inhalation of the vapors of lewisite and phenyldichlorarsine Its pathology in the dogs and treatment with 2,3-dimercaptopropanol (BAL) J. Pharmacol & Exper Therap *87*, 76 (Suppl), 1946

47 Harrison, *H E, Durlacher, S H, Albrink, W S., Ordway, N K*, and Bunting, H.: The treatment by 2,3-dimercaptopropanol (BAL) of the systemic toxic effects of skin contamination with lewisite and phenyldichlorarsine J Pharmacol & Exper Therap *87*, 81, (Suppl), 1946

48. Riker, W. F.: The treatment of experimental arsenic poisoning with the dithiols. J Pharmacol. & Exper Therap. *87*, 66 (Suppl), 1946

49 Riker, W F., and Rosenfeld, G. The effect of 2,3-dimercaptopropanol (BAL) on the whole blood and plasma concentration of arsenic after mapharsen in cats J. Pharmacol & Exper Therap *87*, 72 (Suppl.), 1946.

Current Concepts of Hemolytic Anemias

SOLOMON ESTREN
The Mount Sinai Hospital, New York, New York

AND

WILLIAM DAMESHEK
Tufts College Medical School, and J. H. Pratt Diagnostic Hospital, Boston, Massachusetts

The term "hemolytic syndromes" embraces a variety of clinical conditions in which there is an excessive destruction of red blood cells Although the varying clinical symptomatologies and the major laboratory changes in the different syndromes have been well known for some years, the fundamental mechanisms which produce these diseases are still rather poorly understood This report, after reviewing certain basic principles of physiologic and pathologic hemolysis, will discuss recent methods of study and will summarize current concepts of some of the major hemolytic syndromes

Physiologic Mechanisms

Until the past few years, attempts to estimate the life span of the normal erythrocyte have largely suffered from gross inaccuracies and crudeness of technics Schiødt (154), who reviewed the methods and results to 1938, arrived at a figure of 30 days for the life of the red cell, a value which is undoubtedly too low In recent years, the life span of the normal erythrocyte has been determined by a number of unrelated methods, all of which give a value of some 100 to 120 days Several independent technics may be mentioned

Isotopic Protoporphyrin. Shemin and Rittenberg (156,157) found that feeding glycine containing an isotope of nitrogen, N^{15}, resulted in the production of a protoporphyrin containing this isotopic nitrogen They found also that various other forms of nitrogen (proline, leucine, ammonia) did not substitute for glycine in this process They were able, therefore, to follow the concentration of

66 Longcope, W T, Luetscher, J A, Jr, Wintrobe, M. M, and Jager, V.: Clinical uses of 2,3-dimercaptopropanol (BAL), the treatment of arsenical dermatitis with preparations of BAL J. Clin Investigation *25*, 528, 1946
67. Sulzberger, M B, and Baer, R L: Development and use of BAL J. A M. A *133*, 293, 1947
68 Longcope, W. T, and Luetscher, J A., Jr · Unpublished data.
69 Luetscher, J. A, Jr, Eagle, H, and Longcope, W T.. The effect of BAL on the excretion of arsenic in arsenical intoxication J Clin. Investigation *25*, 534, 1946.
70 Brown, M R, and Hastings, J L · The role of arsenic in the production of alcoholic neuritis Science *105*, 256, 1947
71 Gilman, A · Therapeutic applications of chemical warfare agents Federation Proc *6*, 285, 1946
72 Longcope, W T, and Luetscher, J A, Jr The treatment of acute mercury poisoning by BAL J Clin Investigation *25*, 557, 1946
73 Sollman, T. Manual of Pharmacology. Philadelphia, Saunders, 1942
74 Goodman, A, and Gilman, A *The Pharmacological Basis of Therapeutics* New York, Macmillan, 1941
75 Cohen, A, Goldman, J, and Dubbs, A W · Treatment of acute gold and arsenic poisoning Use of BAL (2,3-dimercaptopropanol, British antilewisite) J A M A *133*, 749, 1947
76 *Ragan, C. and Boots, R H: Treatment of gold dermatitis Use of BAL* (2,3-dimercaptopropanol) J A M. A *133*, 752, 1947
77 Lockie, L M, Norcross, B M, and George C. W · Treatment of two reactions due to gold Response of thrombopenic purpura and granulocytopenia to BAL therapy J A M A *133*, 754, 1947
78 *Ryder, H W, Cholak, J, and Kehoe, R A Influence of dithiopropanol* (BAL) on human lead metabolism Science *106*, 63, 1947.
79 Longcope, W T, and Luetscher, J A, Jr. Unpublished data
80 Germuth, F G, Jr, and Eagle, H The efficacy of BAL (2,3-dimercaptopropanol) in the treatment of experimental lead poisoning in rabbits *J Pharmcol Expt Therap* *92*, 397, 1948
81 Germuth, F G, Jr, and Eagle, H The efficacy of BAL (2,3-dimercaptopropanol) in the treatment of experimental lead poisoning in rabbits Federation Proc *7*, 221, 1948

volunteer, using anti-A serum as the diluting fluid, and noting the number of unagglutinated (donor) red cells within the counting chamber. Callender found that the transfused cells disappeared in linear fashion, with a survival time of about 120 days. Wiener (185) had obtained similar results with M-N transfusions in 1934. It should be noted that the linear nature of this curve, and that of Jope, signifies mathematically that the rate of destruction of red cells is constant and depends on only one factor, namely, the age of the red cells.

MODE OF RED CELL DESTRUCTION

At the end of the 100 to 130 days after the red cell has entered the circulating blood from the site of its production in the bone marrow, it is destroyed. The exact manner of destruction of the red cell is [illegible] occurs as the end res[illegible], rather than because of active phagocytosis of the red cell by the reticulo-endothelial system.

In vitro studies (144) have suggested several means of hemolysis, of which two are of greatest significance:

(*1*) Progressive reduction of the surface area of the cell to a critical point at which the area becomes zero (biconcave disk → crenated biconcave disk → crenated sphere → smooth sphere → explosive hemolysis).

(*2*) Progressive increase in cell volume to a critical point, at which the envelope ruptures (swelling of biconcave disk → sphere → progressively swollen sphere → sudden hemolysis).

In either mechanism, a spherical shape of the red cell on its way to destruction is important; and, *in vivo*, it is known that the spheroidal erythrocyte, the "spherocyte," is also an injured cell on its way to destruction.

In the final analysis, hemolysis must be due to disruption of the envelope of the red cell. Ultimate rupture of this envelope is believed to occur within the reticulo-endothelial system, of which the spleen comprises the greatest single unit. In the normal course of events, there are many factors that combine to cause alterations in the red cell envelope, so that, after some 100 to 130 days, the envelope is sufficiently injured to allow frank rupture during a particular sojourn in the reticulo-endothelial system. Most important

N^{15} in the red cells for months after they had thus labeled the hemoglobin, and found that the life span of the red cell was about 125 days.

Sulfhemoglobin. Jope (102) noted that sulfhemoglobin, once it was formed within the red cells, *persisted long after removal of the* agent responsible for its formation Since the red cells cannot transform sulfhemoglobin, and since the body cannot remove it except by destroying the red cells which contain it, the persistence of sulfhemoglobinemia must be a measure of the survival time of erythrocytes. The presence of sulfhemoglobin does not in itself apparently alter the life of the red cell. Jope studied six miners who developed sulfhemoglobinemia after exposure to trinitrotoluene (TNT), and found that the disappearance curve of sulfhemoglobin was linear, and that the corresponding survival time of the red cell was 116 days.

Transfusion Studies. These have been carried out since 1919 by transfusing cells of a different blood type into individuals and observing the length of time required for the transfused red cells to disappear from the circulation of the recipient. Thus, O cells can be given to A, B, or AB recipients; M cells to N or MN recipients; N cells to M or MN recipients, and Rh negative cells to Rh positive recipients (page 52). Several modifications of the original technic of Ashby (1) have been utilized in such studies:

(*a*) Dekkers (50) transfused M cells to N recipients and determined the presence of donor cells by the use of anti-M serums ("direct agglutination") From his results, he suggested that the *normal life span of the red cell probably exceeds 3 months*

(*b*) Willenegger (190) transfused O cells to A recipients and then used warmed, high-titer, anti-A serum to do red cell counts on the recipients His serum produced hemolysis of the A cells but did not affect the transfused cells, which were of group O; he was therefore able to determine the presence of O cells in the recipient's circulation. On the basis of this method, he believed that the normal life span of the red cell was 100 to 130 days

(*c*) Callender *et al* (14,16) gave group O red cells to healthy group A volunteers and then determined, by means of the original Ashby technic of "differential agglutination," the number of transfused (i e., group O) red cells in the circulation at various times This technic involves performing serial red cell counts on each

HEMOGLOBIN METABOLISM

Iron-protoporphyrin IX-globin or Hemoglobin

↓ reticulo-endothelial system opens a ring

Iron-biliverdinglobin or Hemosiderin

↓ reticulo-endothelial system removes the iron, which circulates as serum iron

Bilirubinglobin or "Indirect" bilirubin

↓ liver cells separate globin

Bilirubin or "Direct" bilirubin

↓ "Direct" bilirubin passes into gut via bile, in large bowel, bacterial flora reduce it to

Urobilinogen (Stercobilinogen and Mesobilirubinogen)

Some re-circulates via liver and bile	Some, refused by liver, is excreted in the urine (1-2 mg /day)	Most is excreted in feces (50-200 mg /day)

iron The remaining compound consists of a molecule of bilirubin which is still firmly attached to the protein of the original hemoglobin, globin. This explains its three characteristics: (*1*) it travels with the serum albumin (globin and albumin behave identically in the ultracentrifuge and on electrophoresis); (*2*) it gives a delayed or indirect reaction in the van den Bergh test, (*3*) it is too large to pass through the kidney filter Bilirubinglobin passes through the liver cells into the bile capillaries; in this passage, the liver cells apparently separate the globin from the bilirubin, so that the bilirubin within bile is "prompt-reacting" or "direct" or "one-minute" bilirubin This form of bilirubin passes readily through the kidneys. From the bile capillaries, the direct bilirubin passes through the bile into the intestinal tract, and in the colon undergoes reduction by the bacterial flora into stercobilinogen and mesobilirubinogen, known collectively as urobilinogen The bulk of urobilinogen is excreted in the feces, some, reabsorbed into the portal circulation,

among these factors are (1) constant buffeting of the red cell in its *passage through the many miles of fine capillaries (149)*; and (2) physical and perhaps chemical changes in the milieu surrounding the red cells during continually recurring periods of stasis ("erythrostasis") in the splenic and other sinusoids (87,88,177). The end point of such forces is the production of irreversible changes in the red cell envelope, with subsequent hemolysis.

The youngest circulating erythrocyte, the reticulocyte, is a relatively thin cell; either because of this or because the youngest cells have been least exposed to the various traumatizing factors within *the body, the reticulocytes are relatively resistant to hemolysis.* The reticulocyte matures within a few days into the normal, biconcave erythrocyte, whose shape and flexibility permit it to pass easily through the networks of narrow blood vessels throughout the body With continuous mechanical buffeting and with constant exposure in the circulation, within the spleen, and possibly elsewhere, to other unknown influences, the biconcave disk gradually becomes more and more spheroidal, until it is frankly a spherocyte. This spherocyte is particularly liable to destruction within the spleen so that denaturation of the wall of the erythrocyte, perhaps by the local erythrostatic factors which so strikingly take place in that organ, finally occurs, following which the contained hemoglobin flows out through the injured cell membrane, leaving a residual *"ghost" of stroma.*

There is no good evidence that actual erythrophagocytosis plays a role in normal hemolysis, or that fragmentation of the circulating erythrocyte, with intravascular disintegration, occurs (149).

METABOLISM OF HEMOGLOBIN

With disintegration of the aged erythrocyte in the reticuloendothelial system, the contained hemoglobin undergoes progressive chemical changes (see scheme) (182). Hemoglobin is a complex of ferrous iron, protoporphyrin IX, and a protein called "globin" The first change consists of a breaking of the ringed structure of the protoporphyrin, so that the hemoglobin gives way to a straight- [illegible] still containing both iron and globin (verdohemo- [illegible]siderin) The iron is then [illegible] called bilirubinglobin. The [illegible] iron which is *split off* circulates with the serum globulin as serum

Hemoglobin Metabolism

Iron–protoporphyrin IX–globin or Hemoglobin

↓ reticulo-endothelial system opens a ring

Iron–biliverdinglobin or Hemosiderin

↓ reticulo-endothelial system removes the iron, which circulates as serum iron

Bilirubinglobin or "Indirect" bilirubin

↓ liver cells separate globin

Bilirubin or "Direct" bilirubin

↓ "Direct" bilirubin passes into gut via bile. In large bowel, bacterial flora reduce it to

Urobilinogen (Stercobilinogen and Mesobilirubinogen)

- Some re-circulates via liver and bile
- Some, refused by liver, is excreted in the urine (1–2 mg /day)
- Most is excreted in feces (50–200 mg /day)

iron The remaining compound consists of a molecule of bilirubin which is still firmly attached to the protein of the original hemoglobin, globin. This explains its three characteristics. (*1*) it travels with the serum albumin (globin and albumin behave identically in the ultracentrifuge and on electrophoresis); (*2*) it gives a delayed or indirect reaction in the van den Bergh test; (*3*) it is too large to pass through the kidney filter Bilirubinglobin passes through the liver cells into the bile capillaries; in this passage, the liver cells apparently separate the globin from the bilirubin, so that the bilirubin within bile is "prompt-reacting" or "direct" or "one-minute" bilirubin This form of bilirubin passes readily through the kidneys. From the bile capillaries, the direct bilirubin passes through the bile into the intestinal tract, and in the colon undergoes reduction by the bacterial flora into stercobilinogen and mesobilirubinogen, known collectively as urobilinogen The bulk of urobilinogen is excreted in the feces; some, reabsorbed into the portal circulation,

ultimately passes via the general circulation to the kidneys, where it is excreted in the urine.

About 1 per cent of the body erythrocytes is destroyed daily This corresponds to a serum indirect bilirubin of 0 0 to 0 8 mg. per 100 cc. of blood plasma, a fecal urobilinogen output of 20 to 180 mg per day, and a urinary urobilinogen output of 1 to 2 mg. per day (123). The bone marrow, attuned to a loss of about 1 per cent of the body erythrocytes daily, delivers a corresponding number of new erythrocytes (reticulocytes) to the circulation. Whenever an exaggerated amount of hemolysis occurs, the amount of serum bilirubinglobin increases, there is an increase in the excretion of *urobilinogen in both urine and feces, and a compensatory reticulocytosis* due to marrow activity occurs. Bilirubin is not found in the urine, because bilirubinglobin is too large to pass the kidney filter; hence the generic term "acholuric jaundice" for cases of hemolytic anemia. Of these changes, the most constant is the increased excretion of urobilinogen, for reticulocytosis may be absent, *and hyperbilirubinemia depends to a certain degree upon the ability* of the liver to handle the bilirubinglobin presented to it

The "hemolytic index" relates the amount of urobilinogen excreted to the amount of hemoglobin in the circulation (123). The normal individual, with 15 5 Gm. of hemoglobin per 100 cc of blood, and some 5,500 cc. of blood volume, excretes about 150 mg of urobilinogen per day, giving a hemolytic index of 10–21 This index is conventionally expressed as the milligrams of fecal urobilinogen excreted daily per 100 Gm of body hemoglobin:

$$\frac{\text{mg fecal urobilinogen daily}}{\text{Gm total body hemoglobin}} \times 100$$

Normally, therefore, 100 Gm of circulating hemoglobin gives rise to *10–21 mg of fecal urobilinogen daily The individual with non*hemolytic anemia should have a urobilinogen excretion proportionate to the amount of hemoglobin which he still has; in other words, the hemolytic index remains unchanged if the anemia is not hemolytic in origin If, however, a hemoglobin of, let us say, 4 Gm. per hundred cubic centimeters, is associated with a "normal" urobilinogen excretion (150 mg. per day), it is obvious that the hemolytic index must be increased, and that hemolysis must therefore play a role in the anemia.

ROLE OF THE SPLEEN

The end stages of normal hemolysis probably occur in the reticuloendothelial system throughout the body. Apparently, within these cells, the injured, aged erythrocyte finally bursts as the result of physical and other forces. It is rarely possible, even in pathologic states, to demonstrate actual phagocytosis of erythrocytes by reticulo-endothelial cells within the blood, marrow, or spleen. Certain investigators have pointed out that blood stagnates within the spleen for long periods and have suggested that this "erythrostasis" may be a factor in hemolysis (87,88,177). Others have suggested that the reticulo-endothelial cells, notably within the spleen, might liberate toxic substances which help to prepare the red cell for destruction (117,117a). Removal of the spleen for nonhemolytic diseases (rupture, idiopathic thrombocytopenic purpura) is followed by a reduction in the amount of hemolysis, presumably because a large part of the reticulo-endothelial system has been removed (123).

There is no outstanding evidence for the presence in normal blood of a circulating hemolysin which produces destruction of red cells. Freeman and co-workers (74,101) found that lipemic serums had a hemolytic action on red cells, and that in certain cases an increase in hemolysis could be produced by increasing the ingestion of fat. The significance of these findings for normal states is not clear. Lysolecithin, a hemolytic agent present in minute quantities in normal human plasma, and increased by incubating the plasma *in vitro*, was suggested by Bergenhem and Fåhraeus (3) as the agent produced within the spleen during erythrostasis and responsible for intrasplenic hemolysis Singer (160,161) could find no increase in lysolecithin in familial spherocytosis; and Foy and Kondi (72) could not produce an increase in hemolysis by the injection of lysolecithin *in vivo* It seems likely that lysolecithin cannot be seriously implicated in hemolysis *in vivo*.

The role of erythrostasis is more difficult to evaluate. The changes in osmosis and pH which occur with erythrostasis within the spleen have been shown to alter the physical characteristics of the red cells toward the direction of spheroidicity (87,88,177). There is little doubt that the spleen destroys red cells which are already damaged when presented to it; and splenectomy is regularly followed, both in

the normal individual and in many patients with various types of hemolytic anemias, by a reduction of the amount of hemolysis

It should be noted that, on the basis of experiments with dogs, Singer (162,165) now believes that splenectomy is not followed by a reduction in the amount of hemolysis; and that the spleen therefore plays little if any part in the normal hemolytic cycle. This work requires confirmation.

Pathologic Mechanisms

LIFE SPAN OF THE RED CELL IN ABNORMAL STATES

In the individual with any type of hemolytic anemia, the life span of the erythrocyte must, by definition of the disease, be abnormally shortened The study of the behavior of normal red cells *within the circulation of such patients, as well as the behavior of the patient's red cells in a normal individual,* provides interesting data Such experiments, known as cross-transfusion studies, are based on various modifications of the technic originally described by Ashby (1). In general, the method entails the transfusion into a given individual of a compatible, although foreign, red cell, which is immunologically different from the red cell of the recipient; and then the determination, by differential agglutination, of the number of foreign cells present in the circulation at successive time intervals A number of different set-ups may be utilized, depending upon the blood group of the recipient (Table I) In all instances,

TABLE I
Set-Ups for Cross-Transfusion Studies

Recipient cells	Donor cells	Agglutinating fluid for red cell counts
A	O	Anti-A
B	O	Anti-B
AB	O	Anti-A, Anti-B
AB	A	Anti-B
AB	B	Anti-A
M or MN	N	Anti-M
N or MN	M	Anti-N
Rh positive	Rh negative	Anti-Rh

the recipient's cells are agglutinated during the red cell count determinations by means of the specific agglutinating fluid. The

donor cells remain unagglutinated, and may be counted directly on the counting chamber. By successive counts, a curve is plotted showing, with the passage of time, the decrease of unagglutinated (transfused) cells This curve therefore measures the life span of the transfused cells in the particular circulation under study.

A few of the results of such studies are:

(*1*) When normal cells are transfused into normal individuals, they disappear in linear fashion in about 120 days (14,16,127,185).

(*2*) When normal cells are transfused into the circulation of patients with chronic hypochromic anemia, they disappear in linear fashion in about 100 days, there is, therefore, no hemolytic factor in this disorder, the variation from 100 to 120 days being within the limit of error of the method (11,14,126a). Patients with hypochromic anemia may thus be used in place of normal human volunteers in cross-transfusion experiments.

(*3*) Normal cells transfused into patients with familial hemolytic anemia of the spherocytic type disappear in a linear fashion in 100 to 120 days When red cells from patients with familial spherocytosis are introduced into a normal circulation, they disappear in an exponential manner within 1 to 19 days (112,29,138). Possible interpretations of these findings are as follows spherocytes are short lived; the defect in familial spherocytosis probably lies within the red cell, the spleen is probably merely the active destroying agent for spherocytes These results will be considered in detail later.

(*4*) Normal cells transfused into patients with acquired idiopathic hemolytic anemias disappear exponentially in from 1 to 15 days (11,63,112,196) When red cells from acquired hemolytic anemias are introduced into a normal circulation, their survival curve may be linear (112,196) These results will be discussed later

(*5*) Abnormal cells of various types, when transfused into a normal circulation, disappear more rapidly than the normal cells of the recipient This has been shown for familial spherocytes (29, 112,138), target cells, sickle cells (15), elliptocytes (21), and the sensitized cells of erythroblastosis foetalis (126)

These studies suggest that the normal manner of destruction of erythrocytes depends upon relatively constant factors, such as the stresses and strains to which the circulating red cells are subjected, since their disappearance occurs in a linear fashion In pathologic

states of hemolysis, however, the destruction of red cells is not linear, but often exponential in type; that is, it depends upon other factors than mere age, such as malformation of the erythrocyte (familial spherocytosis) or some sort of abnormal "hemolytic system" (*certain acquired hemolytic anemias*).

MODE OF DESTRUCTION

Pathologic *hemolysis is not, a priori, merely an exaggeration* of the normal hemolytic mechanisms. In general, three types of excessive hemolysis may be anticipated:

(*1*) Exaggeration of the normal mechanism, because of overactivity of the reticulo-endothelial system (hemolytic hypersplenism; hypersplenic hemolytic anemia) Here, the red cells which are presented to the spleen are normal, but overactivity of this organ produces *exaggerated destruction Hemolysis is intrareticulo-endothelial.*

(*2*) Exaggeration of the normal mechanism, because of excessive vulnerability of the red cells. Here, red cells which are already partially injured by a variety of causes are destroyed by the spleen. The hemolytic anemia is *fundamentally the result of injury* to the cell membrane, and the spleen merely "scavenges" the damaged red *cells presented to it*

(*3*) Appearance of an abnormal mechanism Here, certain types of direct damage to red cells so disrupt the red cell envelope that the cell becomes hemolyzed either directly or with the aid of such normal mechanisms as mechanical trauma, erythrostasis, and splenic activity

Most forms of hemolytic anemia fall into the second group, in *which* some injury to the red cell envelope makes the erythrocyte especially liable to destruction by the normal forces of the body This injury may be heredofamilial (spherocytosis, target cell anemia, sickle cell anemia), or due to a large number of acquired causes. Many *instances of idiopathic acquired hemolytic anemia* probably belong in this group

FRAGILITY TESTS

The *in vitro* disintegration of red cells under various environmental conditions may give a clue to the underlying process in a

case of hemolytic anemia Several *in vitro* fragility tests are recognized.

Hypotonic Fragility Test (17,18,75,142,143). A specimen of blood, as removed at a given moment from a blood vessel, consists of a heterogeneous population of red cells, some old, some young, and many intermediate in age, These differences probably correspond to various alterations in the physical status of the cells, i e, spherocyte, reticulocyte, biconcave disk. The results of the hypotonic fragility test depend directly upon the proportion of the various types of red cells in the blood being examined

When a red cell is subjected to a hypotonic environment, some of the surrounding fluid diffuses into the erythrocyte by osmosis until the tonicity of the red cell and the medium balance. The diffusion of fluid into the cell increases the volume of liquid within the cell, so that the cell becomes less biconcave, and more spheroidal The more the surrounding medium is hypotonic, the more fluid passes into the erythrocyte before balance is reached, and therefore the more spheroidal the red cell becomes. At some crucial level of hypotonicity, the volume of the red cell reaches its limit and the red cell envelope bursts Those red cells which are already spheroidal will require less inosmosis of fluid before hemolysis; hence they break up at only slightly hypotonic levels. Those cells which are unusually thin will require the most inosmosis before hemolysis, and therefore do not hemolyze before marked levels of hypotonicity, approaching that of distilled water, are reached The hypotonic fragility test, therefore, is merely a measure of the thickness and thinness of the red cells in a given blood sample. In normal blood, a few red cells hemolyze in relatively slight degrees of hypotonicity (e g, at 0 50 per cent of NaCl solution), a few only in relatively marked degrees of hypotonicity (e g, at 0.25 per cent of NaCl solution), and the bulk of the cells between these two extremes. Thick cells (spherocytes) break up in slight degrees of hypotonicity, thus, a patient with spherocytic hemolytic anemia is said to show "increased hemolysis" (e g, at 0.78 per cent of NaCl solution). When a red cell population includes many thin red cells (target cells, sickle cells, reticulocytes), the hypotonic fragility is decreased, for such cells do not become hemolyzed until levels of 0 10 per cent or even 0 04 per cent of NaCl solution are reached This correlation between hypotonic fragility and the thickness of

erythrocytes was confirmed by Haden's studies (85) of the red cells of various animals (Table II).

It should be emphasized that there is no *a priori* correlation between this purely *in vitro* test of red cell thickness, and the hemolysis of red cells *in vivo*.

Mechanical Fragility Test. Under standard conditions of mechanical agitation, a given percentage of red cells break up (40). An increase in this percentage has been demonstrated in certain cases of hemolytic anemia, particularly in certain of those having a circulating antibody, and in those due to thermal injury (158). Since

TABLE II

Correlation Between Hypotonic Fragility and Thickness of Erythrocytes

Animal	Diameter, μ	Thickness, μ	Ratio, thickness to diameter	Resistance to saline
Man	7 8	1 84	1:4 2	0 42–0 48
Dog	7 2	1 70	1:4 2	0 50–0 52
Rabbit	6 6	1 84	1:3 6	0 52–0 54
Cat	5 6	1 75	1:3 2	0 60–0 66
Goat	4 0	1 95	1.2 1	0 72–0 74

part of the normal process of hemolysis *in vivo* probably consists of a continual buffeting of the circulating red cells—i e, mechanical trauma—it has been suggested that the mechanical fragility test may parallel the situation *in vivo*, at least to some extent (158). Special varieties of excessive liability to hemolysis by slight mechanical traumas have also been reported in red cells damaged by certain serum antibodies (168,170)

Acid Fragility Test (86,89) In paroxysmal nocturnal hemoglobinuria, the red cells are pathologically liable to destruction by dilute acid solutions (hydrochloric, carbon dioxide). Whether this characteristic explains their hemolysis *in vivo*, has not been established.

Heat Fragility Test The red cells of paroxysmal nocturnal hemoglobinuria are also pathologically liable to destruction by exposure to 37 C (92). Whether this is due to acidification of the medium at this temperature or to other mechanisms has not been determined (124).

IMMUNOHEMATOLOGY

It was Chauffard (19a) who, in 1908, having found an immune hemolysin in the blood serum of a patient with acute hemolytic anemia, first suggested the formation of a new specialty, "immunohematology." The interest in the relationship of immune bodies to the occurrence of hemolytic anemias has become especially keen in recent years. This interest is based upon the existence of hemolytic anemias demonstrably due to circulating antibodies, and the suggestion that many other hemolytic processes, the origin of which is as yet unknown, might be readily explicable if our means for study of immune mechanisms were sufficiently exact

Several varieties of immune bodies may be distinguished. Agglutinins are substances which cause, *in vitro*, the agglutination (clumping) of red cells. Autoagglutinins clump the red cells of the same individual, isoagglutinins, those of another individual of the same blood group, and heteroagglutinins, those of another species. Agglutinins may show maximal activity at icebox temperatures ("cold agglutinins"), or at body temperatures ("warm agglutinins"), although there is often overlapping between temperatures

Hemolysins are substances which, either directly or in the presence of complement, produce hemolysis of red blood cells. Certain substances which produce only agglutination in the absence of complement, cause hemolysis when complement is present; so that "agglutinin" and "hemolysin" may be terms for different activities of the same substance Autohemolysins, isohemolysins, and heterohemolysins are similarly recognized, as well as cold and warm hemolysins Immune hemolysins differ from certain other hemolytic agents (e g, saponin) in requiring complement for their activity

Immune agglutinins and hemolysins are detected by placing in a test tube a red cell suspension (preferably group O), the serum under study, complement (in hemolysin studies only), and a diluent; and then, after allowing time for reaction at the desired temperature (*icebox, room, incubator*), examining for agglutination or hemolysis Successive dilutions of the serum may be prepared to determine the amount (titer) of antibody present

Antibodies have been implicated in the hemolytic processes associated with incompatible transfusions (anti-A, anti-B, anti-Rh) in erythroblastosis foetalis (anti-Rh); in certain paroxysmal hemo-

lytic processes (Donath-Landsteiner antibody, cold agglutinins); and in a number of acquired "idiopathic" hemolytic anemias. The union of the antibody with the red cell envelope is thought to injure the red cell, *thus making it excessively liable to destruction in vivo.* Experiments *in vitro* with various types of agglutinins and hemolysins have provided further demonstrations of these points (40).

Role of the Diluent. It has been found that antibodies may *often be more readily detected by substituting for saline a solution* of albumin or of plasma as the diluting medium (54,135,188). The use of bovine albumin allowed the demonstration of significant titers of agglutinins in five patients with acquired "idiopathic" hemolytic anemia, whereas the use of saline showed no antibodies *at all* (135). *There is a type of antibody which does not visibly* clump or hemolyze red cells, but which "coats" it by adsorption onto the cell envelope. This antibody is known as a "coating" or "blocking" antibody, because it blocks the normal agglutinating reaction between uncoated red cells and specific antibodies; and it is characteristic of these antibodies that they are poorly demonstrable in saline, but readily demonstrable in albumin or serum (188,188a). It is likely that albumin and plasma duplicate the situation *in vivo* more truthfully than does saline and, therefore, that in the nonsaline medium of the blood stream antibodies may be present in high titer, when they are only poorly detected in the ordinary laboratory tests which regularly utilize saline

At least two classes or "orders" of antibodies may thus be recognized: (*1*) those readily demonstrable in saline media, and (*2*) those poorly demonstrable in saline, but readily demonstrable in albumin or serum (97)

Coombs' Antiglobulin Test. Yet a third "order" of antibodies may exist that cannot be demonstrated free in blood plasma but are intimately united with the red cell envelope (97) Their demonstration has been made possible by an ingenious immunologic method (23) If normal human serum, plasma, or plasma globulin is injected into a rabbit, antibodies are produced by the rabbit against the human protein and are present within the rabbit's blood serum. The rabbit serum so produced is spoken of as "anti-human-serum rabbit serum," "antiglobulin serum," or "Coombs' serum This rabbit serum *reacts, in the usual antigen-antibody manner,*

specifically with human serum. It should not react, however, with any other material—such as human red cells—unless human serum is attached to it. In actual practice, for example, anti-human-serum rabbit serum does not react with washed normal red cells; but, when placed in contact with washed red cells from certain cases of hemolytic anemia, it causes clumping of the cells (7). The implication is that such red cells have some human serum factor (antibody factor, or antibody globulin) intimately adsorbed or coated onto them and not removable by washing.

The prime example of such adsorbed antibodies and coated cells occurs in erythroblastosis foetalis (23,96). The red cells of infants affected with this hemolytic disorder, when placed in contact with Coombs' serum, show clumping. This is especially true when the blood plasma of the mother contains blocking antibodies, antibodies which do not exist free in the plasma but become intimately attached to the circulating red cells. Similar clumping of cells in Coombs' antiglobulin serum has been reported in certain instances of acquired hemolytic anemia.

Whether such different types of antibodies are truly present, or whether differing concentrations of antibodies are detectable by varying technics, is not yet clear

INDICATORS OF HEMOLYSIS

Under conditions in which the destruction of red cells becomes increased, whatever the underlying cause, certain changes occur which are common to all hemolytic processes. Conversely, the presence of such changes in a given patient suggests the existence of a hemolytic process. These are changes in the red cells, in the hemoglobin metabolism, in the reticulo-endothelial system (especially spleen), and in the red cell "factory" (the bone marrow).

Red cells. In most cases, destruction of red cells results in a reduction of their number, i e, anemia The hemoglobin of the destroyed red cells is not lost from the body; hence, the anemia is normochromic or sometimes hyperchromic. Exceptions occur in those familial anemias (Mediterranean, sickle cell) in which there is a simultaneous defect in hemoglobin metabolism

In certain of the hereditary and in most acquired forms of hemolytic anemia, the blood contains varying numbers of small, dense spherical cells (spherocytes) Such cells, being thick, are relatively

fragile in hypotonic solutions. *When the blood contains thin red cells (target cells, sickle cells), its hypotonic fragility is relatively decreased, since such cells are relatively resistant to hypotonic solutions.*

Hemoglobin Metabolism. *The hemoglobin–bilirubin cycle becomes quantitatively increased in all hemolytic processes, giving rise to indirect bilirubinemia, acholuric jaundice, and increased excretion of urobilinogen in the urine and feces* If the liver is able to handle all or virtually all of the indirect bilirubin presented to it, it may convert most of the indirect bilirubin into direct bilirubin; there may then be virtually no clinical jaundice and little urinary urobilinogen In such a case, the patient may fail to show jaundice, but the increase in fecal urobilinogen remains unchanged. The latter is therefore sometimes the only definite indication of increased hemolysis, and the possibility of hemolytic anemia cannot be definitely excluded unless this test for fecal urobilinogen is done and the result is within normal limits.

In instances of violent hemolysis, hemoglobinemia and hemoglobinuria may be present.

Reticulo-Endothelial System. Overactivity of the reticulo-endothelial system, the site of removal of excessively fragile erythrocytes, results in the clinical findings of splenomegaly, and sometimes of hepatomegaly and lymphadenopathy.

Bone Marrow and Blood Excessive hemolysis results in compensatory hyperplasia of the bone marrow The marrow pours out large numbers of all types of cells The peripheral blood thus commonly shows, besides anemia. neutrophilic leukocytosis with a shift to immature forms, thrombocytosis; and many immature red cells (reticulocytes, nucleated red cells) In the crises of hemolysis which occur in familial spherocytosis and in acquired "idiopathic" hemolytic anemia, however, a temporary pancytopenia is frequently present (leukopenia, thrombocytopenia, reticulocytopenia, anemia), despite the hyperplastic picture in the marrow

The usual signs of a hemolytic process are pallor and slight jaundice, with splenomegaly Even in those patients with neither clinical nor laboratory jaundice, apparently because the liver is able to handle all the bilirubinglobin presented to it for excretion, the urobilinogen excretion is elevated The only pathognomonic

indication of excessive hemolysis is an increased urobilinogen excretion in the feces (and urine). All other evidences, being indirect, may be lacking.

ROLE OF RED CELLS

On theoretic grounds, it is possible to suggest three mechanisms of hemolytic anemia

(*1*) Intrinsically abnormal red cells Abnormal or injured red cells are preferentially destroyed by the reticulo-endothelial system (they are prematurely old) Patients with red cells which are *fundamentally abnormal—target cells, sickle cells, oval cells, spherocytes* —therefore may show increased hemolysis.

(*2*) Extrinsically abnormal red cells. The normal red cell may be injured by a variety of agents (e g , antibodies in the blood plasma), so that it becomes "prematurely old" and, like other injured red cells, is *preferentially destroyed by the reticulo-endothelial system*

(*3*) Normal red cells with abnormal reticulo-endothelial system. Hyperactivity of the normal hemolytic forces may result in hemolytic anemia in the presence of normal red cells and normal plasma

ROLE OF PLASMA

The blood plasma, within which the red cells circulate, may *house a variety of agents injurious to the red cell, notably, various* antibodies and complement substances Agglutinins, hemolysins, blocking antibodies which circulate within the blood plasma in a variety of clinical conditions, all may injure the envelope of the red cell and thereby make the cell especially liable to destruction by the usual forces of hemolysis. Such plasma agents are important in certain acquired hemolytic anemias.

ROLE OF SPLEEN (RETICULO-ENDOTHELIAL SYSTEM)

Under ordinary circumstances, the reticulo-endothelial system *merely removes from the body aged, worn-out red cells.* Red cells which are abnormal for a variety of reasons are preferentially destroyed by the reticulo-endothelial system. Splenic enlargement in most hemolytic processes seems to be the result of engorgement of the spleen by the damaged red cells undergoing destruction In rare cases, the reticulo-endothelial system, in itself, *seems capable of*

TABLE III

Familial Hemolytic Syndromes

	Spherocyte	Mediterranean	Sickle cell
"Race"	White	Italian, Greek	Negro
Heredity	Mendelian dominant	Mendelian dominant Heterozygous: mild Homozygous: severe	?Mendelian dominant
Cell abnormality	Spherocytes	Target and oval cells	Target and sickle cells
Hypotonic fragility	Increased	Decreased	Decreased
Hemoglobin	Normochromia	Hypochromia	Hypochromia to normochromia
Crises	Common, hemolytic in nature	Rare	Common, multiple thrombotic in nature
Skin ulcers	Rare	Rare	Common
Bone changes	Occasional, slight	Marked	Moderate
Pathogenesis	Production of abnormal cell, the spherocyte, which is excessively liable to destruction by normal mechanisms	Inability to utilize iron correctly, resulting in abnormally hemoglobinated and excessively thin cell (target cell)	Inability to utilize iron correctly, resulting in abnormally hemoglobinated cell; under conditions of hypoxia cell becomes crescent shaped (sickle cell)
Clinical disease	Chronic hemolytic anemia	Chronic hypochromic anemia with hemolytic component in severe cases	Chronic hypochromic anemia with hemolytic component and thrombotic manifestations due to *in vivo* sickling
Effect of splenectomy	Curative (eliminates site of destruction of abnormal cell)	No effect (primary difficulty is hemoglobin abnormality; hemolysis is incidental)	No effect (primary difficulty is *in vivo* sickling and thromboses; hemolysis is incidental)

exaggerated destruction of red cells, giving rise to "hypersplenic" hemolytic anemia.

Classification of Hemolytic States

For the purposes of this report, the hemolytic anemias will be considered under the following headings:

I. Anemias associated with an "intrinsic" abnormality of red cells. Hereditary-congenital hemolytic anemias:
 - (1) With spherocytosis (congenital hemolytic anemia).
 - (2) With target and oval cells (Mediterranean syndromes).
 - (3) With target and sickle cells (sickle cell syndrome).
 - (4) Others.

II Anemias associated with fundamentally normal but injured red cells:

 - A. Injured by extrinsic agent:
   - (1) Physical (burns, roentgen rays).
   - (2) Bacterial (Bartonella).
   - (3) Parasitic (malaria).
   - (4) Allergic (fava bean)
   - (5) Chemical (arsine, phenylhydrazine, sulfonamide, lead).
   - (6) Immunologic.
     - (a) Anti-A, anti-B.
     - (b) Anti-Rh
     - (c) Certain acquired hemolytic anemias.
     - (d) Certain paroxysmal hemoglobinurias.
     - (e) Others?
   - (7) Siderocytic
   - (8) "Toxic" (symptomatic).
 - B Destroyed by overactivity of normal hemolytic mechanisms: "Hypersplenic hemolytic anemia."

Familial Hemolytic Anemias

There are three major hemolytic syndromes which are heredo-familial in character: spherocytic, target cell (Mediterranean) and sickle cell (Table III).

Familial Spherocytosis. This disorder has long been known as congenital hemolytic anemia, congenital hemolytic jaundice, and congenital acholuric jaundice. It is a familial disease, inherited apparently by a Mendelian dominant mechanism, in which the characteristic circulating blood cell is the spherocyte. It is found largely in white individuals, but occasional cases have recently been reported in Negroes (121,152,172). Clinically, the patient shows the usual features of a chronic hemolytic process: pallor, slight jaundice, splenomegaly. The blood shows anemia, spherocytosis, reticulocytosis, and increased hypotonic fragility of the red cells. There is increased excretion of urobilinogen in the urine and feces

In the mildest cases, there are no symptoms, and the only hematologic finding may be a few circulating spherocytes with accompanying increase in hypotonic fragility. In more severe cases, there is moderate anemia with slight jaundice and splenomegaly, a fair number of spherocytes, and a low grade reticulocytosis In severe cases, constant anemia and jaundice, marked splenomegaly, and severe reticulocytosis occur. However, even the mildest cases may, after remaining asymptomatic for many years, show sudden crises of hemolysis at a late age. Several features are of especial importance:

(1) Sudden episodes of rapid destruction of erythrocytes with accompanying exacerbation of all symptoms may occur at any time (hemolytic crises), and at any age, including the newborn (20,91). The precipitating causes for crises are obscure, although crises have occurred following upper respiratory tract infection (31,61) and following compatible blood transfusion (48) Several instances of familial occurrence of crises have been noted (31,49, 155,115,132,99).

(2) The constantly increased amounts of bilirubin presented to the liver and gallbladder result in the precipitation of calcium bilirubinate and the formation of biliary calculi in at least 60 per cent of the cases (98).

(3) Indolent ulceration of the skin about the ankles occurs occasionally, due presumably to stasis and anoxemia at these regions (175,59,113).

(4) Marrow hyperplasia compensatory to the loss of red cells may lead to thinning of the cortex of the bones, but such changes are very rare (13,24)

(5) Splenectomy is followed by clinical cure, but the spherocytosis in the blood persists (125).

The clinical and laboratory findings of the disease can be adequately explained on the basis of the constant spherocytosis, but what produces the spherocyte is problematic. The spherocyte is an "injured" cell, a cell "on its way to destruction"; a cell therefore which is easily and preferentially destroyed within the spleen, i e, the reticulo-endothelial system (40,88). The destruction, by the normal spleen, of excessive numbers of these abnormal red cells gives rise to the usual features of chronic hemolytic anemia. Removal of the spleen results in clinical cure because it eliminates the site of destruction of the injured cells, but there is no true "biologic" cure, for spherocytosis persists after splenectomy.

It is obvious that the spleen itself is not directly responsible for the increased thickness of the red cell, since splenectomy is not followed by any considerable change in red cell thickness Nor, for the same reason, can erythrostasis within the spleen, known to produce spheroidicity of erythrocytes, be implicated causally in this disease. Nor does lysolecithin, an erythrocytolytic substance present in normal blood, and stated to be increased with stasis (e g, within the spleen), but not increased in familial spherocytosis, appear to be the responsible agent

The fundamental etiology of familial spherocytosis seems to lie in an explanation of the spherocytosis. There are at least two schools of thought on the subject: one holds that the spherocyte is due to an inborn defect of red cell production (112,133), the other, that it is due to some hemolytic substance acting upon normal red cells (43). Recent immunohematologic experiments have supported the first thesis—that the spherocyte results from an inherited defect of the erythron When normal blood is given to patients with familial spherocytosis, the introduced red cells survive for 100 to 130 days, and disappear in linear fashion In other words, they behave as they do in normal individuals and do not become spherocytes (29,112,138) But when the red cells of patients with familial spherocytosis are transfused into patients with hypochromic anemia, in whom normal red cells survive normally, they are destroyed exponentially and disappear within 14 to 19 days (29,112,138). The same results obtain both before and after splenectomy. These data indicate that the red cells in familial spherocytosis are intrinsically

defective, so that they are destroyed by the spleen in any circulation containing a spleen; on the other hand, the blood stream and spleen in patients with familial spherocytosis are normal, since they do not destroy normal cells excessively. These findings contrast directly with those in acquired hemolytic anemia, in which normal cells are destroyed rapidly, whereas spherocytes from the patient, when placed into a normal circulation, are usually destroyed normally (196,112) (page 53). At the same time, studies with antiglobulin serum show that the red cells of familial spherocytosis are not agglutinated by Coombs' serum, whereas the spherocytes of certain acquired cases are agglutinated (7). This suggests that in the familial disease the defect which produces the spherocyte is not *due to an adsorbed serum factor, but may well be inborn in the* red cell.

These findings seem to confirm the suggestion that familial spherocytosis is a hereditary error of red cell formation. Further confirmation is afforded by the persistence of spherocytosis after splenectomy, despite the diminution in hemolysis and clinical cure The alternative possibility that the red cell is normal when it leaves the bone marrow but is affected by some toxic substance after it has become a biconcave disk, so that it becomes converted into a spherocyte, has been promulgated especially by one of us (Dameshek). As pointed out by him, the young red cell within the marrow, and the reticulocyte just entering the blood, are normal (not spherocytic) (39) in these patients; the spherocyte may therefore be the result not of defective formation but of the activity of a hemolysin on the reticulocyte or biconcave disk. The occurrence of familial crises in these patients (31,99,155,132,49,115), with the sudden development of extreme degrees of spherocytosis following infection or for no obvious cause, and the occurrence of crises following compatible blood transfusion, suggest that some extrinsic factor may suddenly cause widespread spherocytosis with subsequent hemolysis. It is now well known that spherocytes are not pathognomonic of familial spherocytic hemolytic anemia, but are also found in hemolytic anemias due to drugs and other extrinsic agents (40,43) In guinea pigs (42), monkeys (134), and rats (4) it is possible to produce hemolytic anemias of various degrees of severity by injection of appropriate *amounts of anti-guinea-pig-erythrocyte* (*or anti-monkey, or anti-rat, respectively*) rabbit serum; and the severity of

the hemolysis can be directly correlated with the amount of spherocytosis. Reasoning by analogy, antibodies (hemolysins?) may be responsible for the varying severities of familial spherocytosis. Hemolysins have only occasionally been demonstrated in this disorder (43d), but this fact need not rule out such factors, since our methods for the detection of such antibodies are as yet crude.

It is true that such an explanation must deal with the results of cross-transfusion experiments, which suggest that normal red cells introduced into a patient with familial spherocytosis do not themselves become spherocytes (as might be expected if hemolysins were present). The suggestion has been made that the postulated hemolysin is a highly specific one, acting only upon the patient's own red cells, and therefore does not injure transfused normal cells. There is some evidence that, at least during crises, a circulating antibody may be the responsible mechanism. In a patient with indisputably familial spherocytic hemolytic anemia, transfused red cells were destroyed in the rapid, exponential manner during crisis, at a time when autoagglutinins were also demonstrable in the serum. Subsequent study, after splenectomy, and not during crisis, showed normal linear red cell survival; and antibodies were no longer demonstrable (135a). Antibodies have been reported in other cases of familial spherocytosis in crisis. The occurrence of crises in families suggests the influence of some extrinsic agent, such as a hemolysin perhaps. Crisis, thus, and perhaps the entire disorder itself, may yet be due to some extraerythron abnormality, specific for the patient's own red cells, and giving rise to persistent spherocytosis

The reasons for the occurrence of hemolytic crises, and the hematologic alterations during crisis, are also not completely clear. At least one author believes that the fundamental abnormality in crisis may be acute liver insufficiency and not exaggerated hemolysis (113), but the rapid development of severe anemia in association with jaundice, indirect bilirubinemia, and increasing splenomegaly has long suggested that crises are basically hematologic, and presumably hemolytic, in nature It has generally been taught that the fundamental alteration is a sudden, overwhelming hemolysis. Recent studies, however, have shown that the onset of crisis is characterized by neutropenia and reticulocytopenia in the peripheral blood, a reduction in the amount of serum bilirubinglobin, and maturation arrest of erythroid tissue within the bone marrow

defective, so that they are destroyed by the spleen in any circulation containing a spleen; on the other hand, the blood stream and spleen in patients with familial spherocytosis are normal, since they do not destroy normal cells excessively. These findings contrast directly with those in acquired hemolytic anemia, in which normal cells are destroyed rapidly, whereas spherocytes from the patient, when placed into a normal circulation, are usually destroyed normally (196,112) (page 53). At the same time, studies with antiglobulin serum show that the red cells of familial spherocytosis are not agglutinated by Coombs' serum, whereas the spherocytes of certain acquired cases are agglutinated (7). This suggests that in the familial disease the defect which produces the spherocyte is not due to an adsorbed serum factor, but may well be inborn in the red cell

These findings seem to confirm the suggestion that familial spherocytosis is a hereditary error of red cell formation. Further confirmation is afforded by the persistence of spherocytosis after splenectomy, despite the diminution in hemolysis and clinical cure. The alternative possibility that the red cell is normal when it leaves the bone marrow but is affected by some toxic substance after it has become a biconcave disk, so that it becomes converted into a spherocyte, has been promulgated especially by one of us (Dameshek). As pointed out by him, the young red cell within the marrow, and the reticulocyte just entering the blood, are normal (not spherocytic) (39) in these patients, the spherocyte may therefore be the result not of defective formation but of the activity of a hemolysin on the reticulocyte or biconcave disk The occurrence of familial crises in these patients (31,99,155,132,49,115), with the sudden development of extreme degrees of spherocytosis following infection or for no obvious cause, and the occurrence of crises following compatible blood transfusion, suggest that some extrinsic factor may suddenly cause widespread spherocytosis with subsequent hemolysis It is now well known that spherocytes are not pathognomonic of familial spherocytic hemolytic anemia, but are also found in hemolytic anemias due to drugs and other extrinsic agents (40,43) In guinea pigs (42), monkeys (134), and rats (4) it is possible to produce hemolytic anemias of various degrees of severity by injection of appropriate amounts of anti-guinea-pig-erythrocyte (or anti-monkey, or anti-rat, respectively) rabbit serum; and the severity of

to 50 per cent of the offspring, and is mild in character. However, when two individuals with a mild form of the disease mate, so that two genes for the disorder occur in an offspring, the severest form of the disease, known as Cooley's anemia, results. This concept is based upon detailed incidence studies, which have disclosed that in every case of Cooley's anemia thus far studied both parents have shown the various hematologic abnormalities of the mild condition (30,32,136,166,178). Italians are most commonly affected, especially those who come from Sicily; Syrians, Greeks, and Armenians, less commonly; Turks, rarely (151). Occasional cases have been reported in Negroes (66,171), Chinese (79), and the dark-skinned Jews of Buchara (153). The mild cases are sometimes known as thalassemia minor, and the severe cases (Cooley's anemia) as thalassemia major.

Several degrees of Mediterranean anemia may be distinguished (30,38,191,174). The mildest cases show only hypochromia, the number of red cells being normal or even high (hypochromic erythrocytosis or hypochromic "polycythemia"). The blood smear shows a varying number of unusually thin red cells (leptocytes or target cells), with some oval and elliptic cells, and a few stippled cells. There is a marked variation in the incidence of target, oval, and stippled cells from case to case, some patients showing mostly stippled cells, others chiefly hypochromic ovalocytes, and so on. There are no bone changes, splenomegaly, or clinical complaints in these mild cases. The abnormality is detected accidentally or during planned studies of families. These cases show no evidence of increased hemolysis

In the moderately severe cases, there are moderate anemia, jaundice, and splenomegaly. Target and oval cells are numerous in the blood smear, and the usual evidences of increased hemolysis with compensatory reticulocytosis are present. The compensatory marrow activity may be so marked as to cause moderate bone changes, apparently by expansion of the medulla and marked thinning of the cortex. The extremely severe cases (Cooley's anemia) show striking degrees of anemia, jaundice, and splenomegaly The blood contains an extremely diverse red cell population, with large numbers of oval cells, target cells, stippled cells, and many cells of bizarre shapes. Many reticulocytes and a varying number of nucleated red cells (normoblasts) are seen There is chronic, usually

(138) Owren has interpreted these findings to indicate that the crisis of familial spherocytosis is an acute anemia due to marrow aplasia, and has suggested that some extraneous factor (perhaps related to infection) is responsible However, Dameshek and Bloom, from their studies of seven cases in crisis, suggest that the crisis may rather be the result of sudden overactivity of the spleen (hypersplenism), demonstrated by temporary neutropenia and reticulocytopenia in the peripheral blood despite the plentiful presence of granulocytes and primitive erythrocytes within the marrow (33,36). According to this thesis, crisis is due to a sudden rapid increase in the activity of the spleen, which results simultaneously in an exaggeration of the normal regulatory activity of the spleen on the bone marrow and an increase in the rate of destruction of red cells within the spleen The former action produces a temporary "block" between production of cells in the marrow and their delivery to the circulating blood—hence neutropenia, thrombocytopenia, and reticulocytopenia—while the latter action results in severe hemolysis. As the crisis continues or subsides, reticulocytosis again becomes marked.

Summary. Familial spherocytosis is due to some unexplained hereditary defect, possibly in the erythron, possibly in the reticulo-endothelial system, possibly elsewhere, as a result of which the structure of an abnormally large number of circulating red cells is spherocytic. Since these cells are excessively liable to destruction, a chronic hemolytic anemia results. Splenectomy remedies the clinical disease, because it eliminates the site of destruction of these pathologic erythrocytes, but it does not affect their spherocytic nature

Target Cell (Mediterranean) Anemia. This term embraces a hereditary disorder, occurring chiefly in persons of Italian or Greek ancestry, and to a lesser extent in those of Syrian and Portuguese ancestry It is characterized by a fundamental, inborn error of red cell production, and probably of hemoglobin metabolism as well, leading to the formation of unusually thin red cells which are poorly filled with hemoglobin. The result is the presence of a hypochromic blood picture, associated in severer cases with evidence of increased hemolysis The disorder is definitely hereditary, and appears to be inherited by a mendelian dominant mechanism Mild cases beget mild cases, apparently because they transmit one gene If the patient marries a normal partner, the disorder is transmitted

fusions afford only symptomatic relief (104). Splenectomy has no influence on the ultimate course of the disease, and the hereditary defects persist; but occasional benefit is reported (78), and the operation may be undertaken when the hemolytic process is especially severe or when the very bulk of the spleen impedes mobility

Sickle Cell Anemia. This is a hereditary disorder of the erythron, occurring almost exclusively in Negroes, and characterized by the presence of abnormal red cells in the peripheral blood. The abnormality of [illegible] tion and assumption of a sickle [illegible] to conditions of reduced oxygen [illegible] without anemia, has been found in from 6 to 8 per cent of various groups of Negroes in America (94), and in about 12 per cent of certain African Negro groups (68). The tendency toward sickling is probably inherited by a simple mendelian dominant mechanism. One gene for the trait apparently gives rise to the latent sickling trait. The mechanism by which sickle cell anemia itself is inherited has not as yet been worked out, although it may resemble the situation in Mediterranean anemia (i e, one gene results in the sickle cell trait; two genes, in sickle cell anemia) The disease has rarely been reported in white individuals (80,129,195), and by some is considered absolutely restricted to people with Negro blood (137)

The cells in the circulating blood of sickle cell anemia include both target cells and sickle cells When exposed to conditions of anoxia, most or all of the cells assume the sickle shape. *In vitro*, such a change may be demonstrated merely by ringing a drop of blood, confined between a cover slip and a glass slide, with paraffin; or it may be demonstrated more quickly by allowing the red cells to remain in contact with a culture of Bacillus proteus (163), or by using a solution of the reducing agent, sodium hyposulfite. Sickling has been shown to depend upon the presence within the red cell of [illegible] The cause [illegible] but eryth- [illegible] s the result [illegible] aped sickled [illegible] through the [illegible] peripheral [illegible] ple areas of

severe, hemolysis The marrow activity is marked, and bone changes are striking

Crises are extremely rare in the Mediterranean syndromes. Indolent ulceration of the skin of the ankles is also rare (60). Roentgenograms of the bones in the severe cases show decreased density and widening of the medulla, and thinning of the cortex of the bones (13). These findings are present most typically in the short bones of the hand and in the skull. The skull shows a widened diploe, thinning of the tables, and new bone formation perpendicular to the tables, giving a picture as of "hair standing on end." Similar changes in the mandibles and maxillas, with secondary malformation of the mouth and teeth, occur in the most severe cases.

The fundamental defect in this disorder is not known. Apparently, there is a hereditary defect of red cell development, so that the cells are incapable of utilizing normal amounts of iron to form hemoglobin As a result, a variable red cell population is produced, composed of misshapen, unusually thin cells. The hemoglobin is often confined to the center of the cell and to its rim, giving the cell a bull's eye or target cell appearance. These cells, because of their thinness, are relatively resistant to hemolysis by hypotonic solutions Although they are resistant to hemolysis *in vitro*, they are, like other pathologic cells, liable to excessive destruction *in vivo* This vulnerability may be the cause of the hemolytic process in the severe cases, although an alternative (and perhaps more likely) explanation is the production of bilirubin from heme pigments which are not b

cells are not in them

mias, being found in s

produced experimentally from normal cells by dehydrating the patient, the red cells, or the staining solution (179) However, target cells occur characteristically in large numbers chiefly in Mediterranean and sickle cell anemias.

The incidence of Mediterranean anemia in American Italians has been estimated as about 6 to 8 per cent of the Italian population (32,136,178) The mildest cases require no treatment, the moderate cases do not respond to treatment, and the severest cases usually die before attaining adulthood Iron has no effect on the hypochromia, since the defect seems to lie in an inability of the marrow to utilize iron, and not in the supply of iron to the marrow Trans-

form of familial anemia in which the characteristic cell was hypochromic, microcytic, and relatively resistant *in vitro* to hypotonic hemolysis; there was no clinical hemolysis in these cases.

Stransky and Regala (173) compiled a series of 10 cases of curious familial hemolytic and erythroblastic anemia affecting Filipino natives, in which there was no one typical abnormality of the red cells (i e, no spherocytes, target cells, or sickle cells). The anemia began early in infancy, was hypochromic and hemolytic in nature, and was associated with splenomegaly. Splenectomy had no effect on the course of the disease. The characteristic feature of the blood smear was the presence of large numbers of normoblasts, both before and particularly after splenectomy. Hemolytic crises were often present in these cases.

It is likely that there are other as yet undescribed heredofamilial aberrations of development of the red cells, similar in general pathogenesis to all these disorders. Of especial interest in this regard is the possibility of a familial "siderocytic" anemia. The term "siderocyte" was first used by Grüneberg for red cells containing iron granules in which the iron was in the form of nonhemoglobinous iron (82–84) Originally described as an inherited defect of red cell development in mice, siderocytes have been found in various patients with acquired hemolytic anemias (page 91). We have seen a young man with chronic hypochromic anemia and splenomegaly who, following splenectomy, showed large numbers of siderocytes in the peripheral blood. Splenectomy had little effect on the course of the disease The patient's brother had had, some years previously, an apparently identical disorder, characterized by chronic hypochromic anemia, splenomegaly, brownish pigmentation of the skin, and lack of response to splenectomy. Both brothers showed, in addition to the blood picture and the brownish pigmentation of the skin, very active bone marrow (with no evidence for Gaucher's disease); and our patient had indolent ulcerations about the ankles (34) It seems probable that the presence of siderocytes in such cases indicates a defect of iron-metabolism which allows the occurrence of a certain amount of iron in a nonhemoglobinous form. Like other pathologically formed red cells, these siderocytes may be excessively liable to destruction, thus resulting in a chronic hemolytic anemia.

stasis with further anoxemia and further sickling The sickle cells, furthermore, like other pathologic cells, are exclusively liable to destruction *in vivo*. These facts explain the usual features of the disorder (130,131): (1) multiple thrombotic manifestations (in muscles, near joints, in abdominal organs, heart, brain), with correspondingly diverse symptomatology; (2) chronic hemolytic anemia with pallor, slight jaundice, splenomegaly; and (3) bone changes secondary to the marked reactive hyperplasia of the marrow.

The crises in sickle cell anemia differ from those in familial spherocytosis, since as a rule they are not primarily crises of hemolysis, but apparently the result of multiple coincidental thromboses throughout the body. What initiates these thromboses is unknown; but, once started, they tend to be propagated by the cycle of anoxemia, sickling, thrombosis, more anoxemia, and so on. Clinically, the crises are characterized by pains, fever, weakness, and severe anemia.

Indolent ulcers of the ankle region are not uncommon Marrow hyperplasia may result in bone changes resembling qualitatively those of Mediterranean anemia, but usually of lesser degree (81, 107). The spleen is chronically enlarged, but a curious feature peculiar to this disease is that the spleen, following years of intrasplenic thrombosis and resultant infarction, may become almost completely atrophied in later years.

As regards treatment, transfusions afford only temporary relief Splenectomy is without demonstrable value, except perhaps to lessen in some cases an excessive degree of hemolysis The sickle cell trait does not in itself lead to clinical complaints, but is important from the pathogenetic and hereditary points of view

Other Heredofamilial Syndromes. A number of less common familial defects of the red cell, often associated with hemolytic anemia, have been reported in recent years. Leitner (108) described inherited elliptocytosis in 7 of 8 members of a family, with moderate hemolytic anemia in 2 patients. This author reviewed other reported cases of familial elliptocytosis with hemolysis In 1945, Cooley described another type of familial elliptocytosis affecting 19 of 29 children, although splenomegaly, elliptocytosis, and hypochromic anemia were present in all these cases, no evidences of hemolysis were found (22) Rundles and Falls (150) reported in 1946 another

in many of the other cases of acquired hemolytic anemia listed above. The presence in the blood smear of small, thick, dense cells (spherocytes), together with large, thin, polychromatophilic cells (reticulocytes), is sufficient for a diagnosis of hemolytic anemia; but no inference as to etiology can be made from this finding

It is the large group of cases of "idiopathic" acquired hemolytic anemia which has provoked the greatest interest in recent years. As a result of improved clinical and laboratory methods, it has been possible to determine the etiologies of a number of these "idiopathic" cases, and to surmise the pathogenesis of others. From this somewhat heterogeneous group there have therefore been separated a few varieties of acquired hemolytic anemia; notably, transfusion reactions, erythroblastosis foetalis, symptomatic hemolytic anemia, hypersplenic hemolytic anemia, and perhaps siderocytic hemolytic anemia The bulk of the interest, however, has been evinced in the relationship of immunologic mechanisms to injury of the red cell envelope *in vivo*; and it has been possible to incriminate immunologic factors in many hitherto "idiopathic" hemolytic processes. In fact, it has seemed likely that the majority of such cases will be explained, as our methods for the demonstration of immune antibodies become increasingly sensitive

Agglutinins, hemolysins, and "coating" antibodies have been found in the serums of numerous individuals with hemolytic anemias. As previously outlined, "agglutinin" is the term given to an antibody which causes the clumping of red cells *in vitro*; "hemolysin," to a material which, in the presence of complement, causes dissolution of the red cell envelope; and "coating antibody," to a more elusive material which is adsorbed to the cell membrane, but cannot be discovered in the blood plasma This latter antibody is demonstrated by showing that the "coated" red cell can no longer be agglutinated by specific agglutinins. Serum agglutinins can be demonstrated by mixing serum with a suspension of red cells; hemolysins, by mixing serum, red cell suspension, and complement; and coating antibodies, by more complicated technics, notably the use of Coombs' antiglobulin serum It is probable that all these antibodies may sometimes be present in the body and yet not be demonstrable by our relatively crude technics The use of albumin, serum, or plasma, instead of saline, as a diluting medium affords

Acquired Hemolytic Anemias

Hemolytic anemia due to a variety of physical and chemical agents has been described, including roentgen ray irradiation, physical agents (burns), bacteria (Clostridium welchii, Bartonella), protozoa (malaria), chemicals (lead), drugs (phenylhydrazine, neoarsphenamine, sulfonamides), and allergic agents (fava bean). These anemias are characteristically acute, even fulminating, hemolytic processes, and are directly attributable to the foreign or extrinsic agent. Apparently, the previously normal red cells in such individuals are directly injured by the offending agent; and, since

[illegible] lestruction *in vivo*, hemolysis [illegible] have been reported of hemo- [illegible] administration (71,148), and of hemolysis following severe burns (10,158,159). Hemolysis has also been reported following the inadvertent introduction of distilled water into the blood stream during transurethral resection of the prostate; direct hypotonic hemolysis of circulating red cells is presumably responsible for the hemolytic process in such instances (26).

The course of events has been particularly well demonstrated in [illegible]

fragmented, budding red cells, cells which were small and dense (i e, spherocytes), and which could be produced experimentally by heating blood *in vitro*, and by injecting heated blood *in vivo* (158, 159) These cells showed evidence of the injury produced by the heat to the cell membrane, and there were corresponding increases in the hypotonic and mechanical fragilities of the cells. Hemolytic anemia was therefore to be expected in such patients, as the result of the thermal production of a large number of pathologically fragile erythrocytes In those patients in whom hemolysis occurred, the damaged cells frequently broke down within the blood stream, and hemoglobinemia and hemoglobinuria resulted

It should be noted that the nonspecific nature of the spherocyte —a cell which is merely an injured cell, a cell "on its way to destruction," and not a pathognomonic cell (as was previously held) of congenital hemolytic processes—is evidenced by its occurrence not only in patients with hemolytic anemias following burns but also

versal" donors is therefore t [illegible]
(e g , anti-A of 1:256) sho [illegible]
donors should not be used. [illegible]
of unpooled plasma. This precaution is of particular importance in patients with hemolytic anemia, who already have injured red cells in their circulation. The neutralization of the anti-A and anti-B agglutinins by the addition of A and B substances to the blood before transfusion of a group O blood does not completely eliminate the incidence of hemolytic reactions (176). It must be admitted, however, that occasionally the transfusion of even frankly incompatible red cells (e g , of B cells to an A recipient) does not result in hemolysis (57). The reasons for this phenomenon are completely obscure.

For all practical purposes, the agglutinogens M, N, and P may be excluded from consideration as possible causative agents in transfusion reactions

Anti-Rh and Anti-Hr Antibodies. In addition to the agglutinogens known as A, B, M, N, and P, human red cells contain certain agglutinogens known collectively as the Rh–Hr system. There are three Rh agglutinogens known respectively as C, D, and E.

The older terminology, Rh and Hr, is being superseded by a more fundamental terminology according to the genetic hypothesis of Fischer (145,146). The terms Rh and Hr may be retained for general clinical usage; but the use of the newer terminology in all but the most general discussion seems indicated The relationship between terminologies is as follows:

$Rh' = C$	$Hr' = c$
$Rh_o = D$	$Hr_o = d$
$Rh'' = E$	$Hr'' = e$

The genetics of the Rh antigen are such that, if a given chromosome lacks one of these factors, it must possess in its stead a related factor designated, respectively, as c, d, or e This curious reciprocal relationship has been emphasized by using the term "Hr" for the antigens which are present within a chromosome when the corresponding Rh antigen is absent Each chromosome must therefore possess C or c, D or d, and E or e. Since there are two such chromosomes (one from each parent) in each individual, each red cell must harbor two sets of three of these agglutinogen factors. The make-up of a given individual, for example, may be CDE/CDE, i e , the Rh factors are all present in each chromosome; or CDe/cDe,

a greater chance for the demonstration of whatever antibodies are present.

The mere presence of an antibody during a hemolytic process does not necessarily demonstrate a cause and effect relationship. In many cases, a direct etiologic relationship can be shown; in others, it may reasonably be inferred.

Antibodies Anti-A and Anti-B. It is known, although not explained, that individuals whose red cells contain agglutinogen A have agglutinin anti-B in their serum; and those whose red cells contain agglutinogen B have agglutinin anti-A in their serum. The introduction, therefore, into a group A patient of group B red cells results in an immunologic reaction between the recipient's anti-B and the donor's B (i e, *between recipient's plasma and donor's red cells*), with agglutination of the introduced red cells. Agglutinated red cells have injured envelopes, and are therefore abnormally [illegible] characteristically [illegible] it *hemoglobi-* [illegible] accompanies the initial stage of massive hemolysis. Later, renal shutdown occurs, due probably to the deposition of large amounts of hemoglobin pigment (acid hematin) in the renal tubules. *It has recently been* demonstrated that the development of renal shutdown in these cases may depend to some degree upon pre-existing dehydration (90,197, 106).

The reverse phenomenon, clumping of the recipient's red cells by the donor's plasma (in the case above, of A by anti-A), is usually minimized or largely prevented by the rapid dilution of the transfused plasma in the recipient's blood stream. This has been the basis for the use of so-called universal donors, i e, group O individuals, whose cells contain no A or B agglutinogen and are therefore not clumped by anti-A or anti-B serum. Individuals belonging to group O have anti-A and anti-B agglutinins in their plasma, however. Some have an unusually high titer of either one or the other agglutinin, with the result that, despite the dilution mechanism, suf- [illegible] transfusion to cause ag- [illegible] (A, B, or AB) red cells [illegible] titer, mild or even moderately severe hemolytic reactions may result (58,198), and death has occasionally occurred (128). The indiscriminate use of "uni-

cells of the fetus, produce hemolytic anemia *in utero,* at birth, or postpartum. First pregnancies are rarely followed by hemolytic disease in the newborn, and when it does occur there is usually a history of previous sensitization by transfusion or injection of Rh-positive blood (111). Even in subsequent pregnancies, the disease occurs in only a small percentage of Rh-negative mothers bearing Rh-positive children, probably because of variations in individual productivity of antigen. The actual incidence of erythroblastosis foetalis lies somewhere in the range between 1 case per 400 deliveries (9) and 1 case per 150 deliveries (51,52).

Hemolytic Disease of the Newborn. Hemolysis of the fetus's red cells through an immunologic mechanism such as described above gives rise to hemolytic disease of the newborn. Because the neonatal hematopoietic system is extremely labile and overly responsive to stimuli, such infants show in their circulating blood large numbers of immature blood cells poured out by the marrow and including, notably, nucleated red cells ("erythroblasts"). The disease has therefore long gone under the name of "erythroblastosis foetalis." Its fundamental nature as a hemolytic process has been demonstrated beyond doubt both by study of the excretion of bile pigments (37), and by the determination of the survival time of Rh-negative cells in the infant's circulation (126). Its harmful effects result not only from the presence of anemia, but also from the various types of injury brought about by the agglutinated red cells in various capillaries and sinusoids and by the liberated hemoglobin products. The resulting damage may affect the liver, the kidneys, or the brain. If the disease is most marked *in utero,* fetal hydrops may occur. If it is most marked at and after birth, severe hemolytic anemia with icterus gravis neonatorum follows. In those children who recover, there may be permanent brain damage associated pathologically with jaundice of the brain (kernicterus).

Erythroblastosis foetalis occurs as the end result of any immunologic system in which the mother lacks an antigen which is present in the fetus In 92 per cent of the cases, it is the Rh complex which is involved: under such circumstances, the mother is Rh negative, while the father and fetu[illegible]
the mother is Hr negative, while t[illegible]
both mother and child are grossly [illegible]

with both Rh and Hr factors present on each chromosome; and so on.

These formulas represent the theoretic genetic make-up of red cells. In practice, it is often impossible to determine the constitution of each chromosome separately, nor is it necessary. By using appropriate agglutinins (blood serums), cells may be classified as "Rh positive," "Rh negative," "Hr positive," etc. Depending upon the strength and specificity of the serums available for testing, it may be possible to break down the large classification "Rh" into C, D, E, and their combinations, and similarly for Hr. In general, those individuals who lack a given factor in their red cells may become sensitized to that factor. For example, Rh-negative individuals may be sensitized by exposure to Rh-positive cells; Hr-negative individuals, by exposure to Hr-positive cells; C-negative individuals, by exposure to the factor C. These generalities are subject to variations in antigenic strength, dosage of sensitizing antigen, time of exposure, and other factors (110,187)

Those individuals whose red cells lack the Rh factors do not naturally harbor a corresponding antibody (anti-Rh) in their plasmas; but they are capable, if artificially sensitized by the Rh antigens, of producing such antibodies The two stimuli which produce sensitization are (1) transfusion with Rh positive cells, and (2) pregnancy with an Rh positive fetus

The first transfusion with Rh-positive blood does not cause a hemolytic reaction, but merely brings into play the recipient's antigenic capabilities so that he produces anti-Rh antibody, which then circulates in the blood stream. The subsequent introduction of Rh-positive cells into the circulation may result in the union of antigen (in the introduced red cells) and antibody (in the recipient's serum), with resultant clumping of the introduced cells and the usual subsequent course of an intravascular hemolytic process In the case of an Rh-negative woman, pregnancy with an Rh-positive fetus may have the same effect as introduction of Rh-positive cells by transfusion, namely, the production of anti-Rh antibodies. If Rh-positive blood is subsequently given to the mother, either during pregnancy or parturition, or at some later date, a hemolytic reaction may result If in a subsequent pregnancy the fetus is Rh positive, the maternal antibodies may pass over the placenta into the circulation of the fetus and, by clumping the red

most workers. It is likely, however, that results in the mildest cases are not sufficiently altered by the use of Rh-positive cells to show a statistical change.

The evidence for a possible "protective factor" in Rh-positive blood which would make its use desirable in cases of erythroblastosis foetalis is at present meager, and so far such a possibility must be considered unproved (5,6).

Exsanguination–Transfusion Since continued destruction of red cells by plasma antibodies is harmful, removal of the antibodies would seem to be of potential benefit. It is questionable whether it is possible to prevent the formation of antibody in the mother by giving her reticulo-endothelial system "something else to do"—i e., competition of antigens—by means of typhoid-pertussis vaccine injection so that antibodies against these disorders would be produced. It was suggested, therefore, that removal of the infant's blood might eliminate both antibody and affected cells, and allow easier recovery. Technics of bleeding the infant and introducing Rh negative whole blood simultaneously were therefore devised, and it has been found possible to "exchange-transfuse" the newborn successfully. Wallerstein (181) originally removed the infant's blood from the longitudinal sinus through the anterior fontanel, and introduced the new (Rh-negative) blood into a cannulated arm vein. Weiner *et al* (189) prefer to allow the infant to bleed from an incised radial artery, while the Rh-negative blood is given into an ankle vein Diamond (53) and Vogel (180) have utilized the umbilical vein both as a site for exsanguination and as the route of transfusion: they cannulate, with a special plastic tube, the umbilical vein and thence the vena cava and withdraw blood and give Rh-negative blood through the one cannula by means of multiple syringes By all these methods, it has been found possible to replace some 85 to 95 per cent of the baby's Rh-positive cells with Rh-negative cells. In appropriate cases, the procedure has saved the infant's life.

Early Delivery In combination with the above technics, early delivery of the viable infant (e g, in the eighth or even seventh month) is often recommended, in order to remove the child from further exposure to the antibodies passed over by the mother (2, 12,55,109).

The treatment of choice, therefore, combines early delivery with

[illegible] C (e g., is cDE/cDE), whereas [illegible] ionally, the mother is of group O, while father and fetus belong to group A (or group B) (95,140, 141). The mechanism and the resulting pathologic findings are largely identical in all cases, although it is likely that A and B sensitization is milder than Rh sensitization.

The treatment of the affected child has undergone successive modifications. The fundamental purpose of treatment is to tide the infant over the period of continued hemolysis until the hemolytic antibodies have disappeared. The hemolytic process is the result of the simultaneous presence in the child's circulation of Rh-positive red cells and anti-Rh agglutinins (passively transferred from the mother via the placenta). So long as these two factors are present, hemolysis continues. Several approaches to therapy have been made.

Blood Transfusion. Originally, whole blood of the same blood group as the child's, but without reference to the Rh factor, was given, because the child, being anemic, obviously required red cells. Before the Rh "situation" was clear, and when no attention to the Rh status of transfused red cells could be given, aggravation of hemolysis often occurred More recently, the use of Rh-negative cells has prevented agglutination of the introduced red cells by the agglutinins, and has allowed alleviation of the anemia without increasing the amount of hemolysis. It was subsequently shown that Rh-negative cells survived normally in the affected child's circulation, as opposed to Rh-positive cells (126). The use of Rh-negative blood has therefore become a standard procedure in the treatment of this condition.

Certain workers maintained, however, that there was no harmful effect from the use of Rh-positive blood, and that it might even be beneficial, since the introduction of sufficient antigen to unite with the antibodies present might eliminate the antibodies more rapidly (44,45). (The amount of antibodies is fixed in any given case, being transferred passively from the mother and not manufactured by the child) However, since it is the clumping of red cells and the deposition of bilirubin which lead to damage, and not the anemia as such, aggravation of hemolysis by providing more red cells for the agglutinins to react with seems an unreasonable procedure. The use of Rh positive cells has been interdicted by

ing reasons: (*1*) There are neither historical nor physical or hematologic evidences for spherocytosis in the patient's family. (*2*) Spherocytes are not pathognomic of a hereditary trait, but have been found in hemolytic anemias due to various external agents and have been produced experimentally. (*3*) There are certain immunohematologic differences between these cases and definite cases of familial spherocytosis. (*4*) [illegible] on these cases than on the [illegible] occurs after splenectomy, the [illegible] i e , spherocytosis does not persist. It has accordingly been postulated that the spherocytosis in these cases is due to some well-defined extrinsic agent, differing in this respect from the generally assumed hereditary abnormality as seen in familial spherocytosis

Immunohematologically, the cases of acquired hemolytic anemia have been studied for abnormal serum antibodies, have been reproduced experimentally, and have been subjected to cross-transfusion experiments. The serum of the patients has often revealed the presence of immune autoagglutinins and autohemolysins Chauffard and co-workers (19a–b) were the first to demonstrate (1908,1909) isohemolysins in the blood of two patients with acquired hemolytic anemia. Since then, various types of serum antibodies have been found in such patients, including "cold" and "warm" autoagglutinins (8,27,43a,62,105,114,147,186), and autohemolysins and isohemolysins (27,41,43,43b,43c,46,67,167). Improvement of technics of demonstration has allowed increased discovery of serum antibodies in recent years. That these technics are still far from perfect is evident from the occasional case in which, although no agglutinin or hemolysin can be demonstrated *in vitro*, transfusion of compatible cells to the patient is followed by their pathologically rapid destruction *in vivo* (64).

It is reasonable to infer that the circulating serum antibodies in these patients injure the red cells and therefore allow their easy destruction; that is, the antibodies are the cause of the hemolytic anemia Experimentally, it has been possible to produce hemolytic syndromes corresponding to acquired "idiopathic" hemolytic anemia, by the injection of appropriate anti-red cell antibodies. In 1938, Dameshek and Schwartz (42) produced such hemolytic anemia in guinea pigs by the injection of anti-guinea-pig-erythrocyte rabbit serum By varying the dosage of antiserum injected, it was possible

exsanguination–transfusion at birth in selected cases. The latter procedure is imperative when the child is born with cells which agglutinate spontaneously in albumin or in Coombs' serum. It is elective if, although the anti-Rh titer of cord blood is high, the cells are not coated (i e., there is little or no "blocking" antibody), and the appearance of the child is fairly good. If the antibody titer is low and the appearance is good, active treatment may be delayed. In many cases, simple transfusion of Rh-negative blood is enough to effect a cure. In some cases, transfusion may become necessary at the age of 3 to 4 weeks, when anemia, nonhemolytic in type, frequently develops.

With these measures, infant mortality due to erythroblastosis *foetalis has fallen from a level of some 40* per cent *in 1942 to about* 10 per cent in 1947 (52).

Certain Acquired "Idiopathic" Hemolytic Anemias. The disorders discussed above are acquired hemolytic anemias demonstrably due to or associated with immunologic mechanisms. Certain other types of acquired hemolytic anemia lend themselves to similar explanations It is this group of acquired hemolytic anemias, in which no obvious causative extrinsic agents can be implicated, which has evoked greatest interest in recent years That this is a rather heterogeneous group is demonstrated by the separation from it of certain subgroups, as their etiologic mechanisms become known. In this way, "symptomatic" hemolytic anemia, "hypersplenic" hemolytic anemia, and other types have been successively separated Most of the cases, however, retain the generic classification of acquired "idiopathic" hemolytic anemia.

Clinically, the patient shows the usual features of a more or less acute or subacute hemolytic process: weakness, highly colored urine, pallor, jaundice, splenomegaly. Hemolytic crises are common. Hematologically, the blood smear often shows the pathognomonic combination of small spherocytes plus large polychromatophilic red cells (i e, injured cells and regenerating cells), and the features of compensatory marrow hyperplasia (granulocytosis with immature forms) are present. The urinary and fecal urobilinogen excretions are high.

The study of the etiology of these "idiopathic" cases has been intensified in recent years The doctrine that they are all latent cases of familial spherocytosis is no longer tenable, for the follow-

anemia, it is postulated that some sort of hemolytic system is present which indiscriminately injures all types of red cells (patient's and donor's), and the injured red cells are then readily destroyed in the spleen The spherocytes of the patient himself, however, may become normal when removed from this "hemolytic system," suggesting that the sensitizing agent is neutralized under these conditions.

This reversal of antibody–red cell union *in vivo* is not so curious as it may at first seem If group A erythrocytes are mixed with anti-A serum in the test tube, and these "sensitized" cells transfused into a normal recipient, they show a normal linear survival curve with a survival time of some 100 days (112). Apparently, the sensitization of erythrocytes by serum antibodies is, at least under certain circumstances, reversible by vague *in vivo* mechanisms. Acquired hemolytic anemias in which there is exponential destruction, both of foreign cells transfused into the patient and of the patient's cells transfused into a normal individual, have not yet been reported.

Obviously, such a situation is the incompatible transfusion, for example, of B cells to an A recipient, and of A cells to a B recipient; but this artificial set-up is not relevant to the discussion

Confirmation of the belief that the red cells in acquired "idiopathic" hemolytic anemia are injured by a serum antibody is afforded by the results of antiglobulin testing (Coombs' test) (7, 65,135) When washed red cells taken from these individuals are placed in anti-human-serum-rabbit serum, they become clumped (positive reaction). Since, as has been discussed, the rabbit serum combines specifically and only with human serum, it must be concluded that some serum substance is intimately affixed to the red cells, and that the contact is so intimate that it cannot be reversed by washing This substance might be a serum agglutinin or hemolysin, the effect of which on the red cell is to injure the envelope and produce the spherocyte. It may be recalled that the spherocytes of familial spherocytosis, on the other hand, are not clumped [illegible] rum substance [illegible] nolytic anemia [illegible] ocytosis, where, except perhaps in crisis, the spleen merely removes previously injured red cells In the acquired cases, the spleen may be responsible,

to vary the resulting syndrome from one of mild hemolysis to one of severe, explosive, intravascular hemolysis and death. The blood smears of the affected animals showed the characteristic spherocytosis plus reticulocytosis; and the more severe the hemolysis, the greater the spherocytosis The suggestion naturally followed that similar immune bodies might be responsible for various forms of hemolytic anemia in the human being.

It is probable, therefore, that a causative relationship exists between circulating autoantibodies and red cell hemolysis. The antibody in these cases presumably unites immunologically with the red cell, injuring its envelope, producing a spherocyte, and rendering the cell more readily destructible by the usual body mechanisms for hemolysis Evidence that the red cell is actually injured is afforded by the results of *in vitro* and *in vivo* tests. *In vitro*, the red cells of patients with circulating serum autoagglutinins are clumped by the patient's serum. Such clumped red cells have been shown to be unusually vulnerable to destruction, as by mechanical means Actual lysis of such cells may occur, either by the action of complement, or through mechanical, chemical, or [illegible] tion of transfused compatible red cells (8). It is of interest in this connection that hemolytic anemia does not occur when serum antibodies are not homologous in nature (for example, in the heterophile antibodies of infectious mononucleosis).

Cross-transfusion experiments in patients with acquired idiopathic hemolytic anemia have helped in the study of these diseases, and in their differentiation from cases of familial spherocytosis It may be recalled that in the latter normal red cells survive normally in the circulation of patients with familial spherocytosis, whereas the familial spherocytes are preferentially destroyed in the normal circulation The reverse phenomena have been found in the acquired cases: transfusion of red cells into these patients results in a rapid, exponential destruction of the introduced red cells When red cells from the patient, however, are transfused into normal circulations, they may show a normal life span and a disappearance in the normal linear fashion. It has therefore been concluded that these are two, fundamentally separate, disorders In acquired hemolytic

at least in part, for the production of the serum antibodies; and splenectomy is often followed by disappearance of spherocytes and serum antibodies, and subsequent return of the blood to normal Cure in such cases may be the result of cessation of immediate hemolysis by removal of a large portion of the reticulo-endothelial system where antibodies are produced, and of removing the organ (spleen) which selectively destroys spherocytes. However, spherocytosis and rapid destruction of erythrocytes may persist after splenectomy, despite clinical improvement. This fact is probably correlated with the persistence of antibodies for months or years following splenectomy. It is possible, too, that some of the hemolysis in these cases is intravascular, and not within the reticulo-endothelial system alone; splenectomy would have little effect in such types of hemolysis

It is therefore possible to distinguish a group of cases of acquired hemolytic anemia which are probably immunologic in origin, and which respond fairly well to splenectomy, about 60 per cent of such patients making a complete recovery after the procedure

Paroxysmal Hemoglobinurias. Of the four major types of paroxysmal hemoglobinuria (Table IV)—syphilitic, cold agglutinins, nocturnal, and exertional—immunologic mechanisms have been implicated in the first three "Exertional" or "march" hemoglobinuria is thought by some to be due to a defect of the kidneys, whereby intrarenal hemolysis is favored by exertion in an erect or lordotic position, and hemolysis is confined to the kidneys. According to this concept, it is really an abnormality of the kidneys, and not a hematologic disorder at all (194) Chronic hemolytic anemia does not occur (76)

The pathogenesis of the other three paroxysmal hemoglobinurias, however, are true hematologic abnormalities of fundamental interest

Paroxysmal Cold Hemoglobinuria (Cold Agglutinins) In certain infections, particularly in primary atypical pneumonia, the serum may contain a large concentration of abnormal antibodies which may cause agglutination of the patient's own red cells (69,70,77, 170). These antibodies are characteristically most active at low temperatures and are therefore known as "cold" agglutinins *In vitro*, they cause clumping of red cells at icebox temperatures, and the clumped red cells are highly susceptible to disintegration (hemolysis) by slight mechanical traumas *In vivo*, the cold agglutinins

TABLE IV. The Paroxysmal Hemoglobinurias

	Paroxysmal cold hemoglobinuria (syphilis)	Paroxysmal cold hemoglobinuria (cold agglutinins)	Paroxysmal nocturnal hemoglobinuria (Marchiafava-Micheli)	Paroxysmal exertional hemoglobinuria
Etiology	Tertiary syphilis	Infections (virus)	Unknown	Unknown
Exciting agent	Cold followed by warmth	Cold	Sleep, horizontal position	Exertion in lordotic or erect position
Red cell	Normal	Normal	Acts as if a substance were affixed to it ("sensitized")	Normal
Serum	Contains Donath - Landsteiner hemolysin, which acts when exposed to cold followed by warmth	Contains autoagglutinin, which is most active at cold temperatures	Normal	Normal
Pathogenesis	Syphilis produces abnormal serum antibody which affixes itself to the red cell envelope in the cold, altering the envelope proteins so that, on subsequent warming, the red cells hemolyze	Certain virus infections produce serum antibodies which cause clumping of the red cells, especially in the cold, clumped red cells, having damaged envelopes, are easily destroyed *in vivo*	Red cells are already "sensitized" by an unknown mechanism, for, in presence of complement (and slight acidity, such as during sleep) they hemolyze	Possibly, local stasis and thence destruction of red cells in kidneys
Physical examination	Tertiary syphilis	Nothing, or, pallor, slight jaundice, splenomegaly	Pallor, jaundice, splenomegaly	Normal
Blood picture	Paroxysmal hemolytic anemia (slight)	Acute hemolytic anemia	Chronic hemolytic anemia, no spherocytes	Normal
Red cell fragility				
Hypotonic	Normal	Normal	Normal	Normal
Acid	Normal	Normal	Increased	Normal
Heat	Normal (if cold followed by heat, cells excessively fragile)	Normal	Increased	Normal
Other data	—	Raynaud's phenomenon	Constant hemosiderinuria leads to chronic glomerulonephritis and uremia	Occurs typically in young healthy men, such as athletes and soldiers

been suggested, therefore, that they are destroyed more easily when the blood and body tend to become acid, as they do during sleep.

Studies by Jordan (103) and by Ham (86) and Ham and Castle (88) have attempted to elucidate the erythrocyte abnormality. Jordan has postulated a parallelism between this disorder and the paroxysmal hemoglobinuria of syphilis. In the latter, a serum substance (antibody or amboceptor) unites with the red cells in the cold. Although the red cells now look normal, they hemolyze readily in the presence of heat and complement:

Amboceptor (serum) + cells + cold = sensitized red cells
Sensitized cells + complement + 37 C = hemolysis.

Jordan believes that in Marchiafava-Micheli disease the red cells in the circulation are already sensitized, i e, that for some unknown reason amboceptor is already affixed to them. No antibody can be demonstrated free in the serum of these patients, but the red cells hemolyze readily in the presence of complement and acid:

(Sensitized) cells + acid + complement = hemolysis
(Sensitized) cells + complement + 37 C. = hemolysis

It has been suggested that the lysis of these cells at 37 C. ("heat fragility") is due not to the temperature but to the production of acid at the temperature; so that this is actually another method of performing the acid fragility test.

Further studies of the red cells and their hemolysis under controlled conditions of acidity, heat, and clot formation have been made (103).

Role of Acid plus Heat. The red cells plus complement show varying degrees of hemolysis with variations in acid and heat When exposed both to heat and to carbon dioxide (a source of acid), hemolysis was intense When exposed only to heat, hemolysis was moderate; and an equivalent amount of hemolysis could be produced by substituting carbon dioxide at room temperature for heat When the cells were left at room temperature without carbon dioxide, no hemolysis occurred It was further found, however, that the cells were more susceptible to these agents before, during, and immediately after a clinical paroxysm of hemolysis; conversely, that when such susceptibility was great, a crisis was present or imminent. The inconstancy of these data, however, makes exact predictions impossible

also cause sensitization or clumping of circulating red cells, and the clumped red cells break up within the blood stream. Agglutination and subsequent hemolysis of red cells occur most markedly on exposure to the cold; but, when *the titer of autoagglutinins is high*, the agglutinins often show some activity at room temperature, and hemolysis may occur without exposure to cold Cold agglutinins have been reported in titers as high as 1:2,000,000 (184). Hemolysis is severe, and may result in severe anemia. In addition, it may be difficult to group and cross-match blood for transfusion, since cold agglutinins are isoantibodies as well as autoantibodies, and therefore clump all human red cells, including those of group O. Typing such blood is made possible by performing the test at body temperatures, and transfusion reactions may be obviated by placing hot water bottles about the transfusion bottle

Paroxysmal Cold Hemoglobinuria (Syphilis). Donath and Landsteiner in 1904 were the first to describe a form of circulating immune body capable of sensitizing red cells so that they were easily destroyed with the production of a hemolytic process (56). This antibody, which sensitized the patient's red cells in the cold so that, on subsequent warming, they were hemolyzed, was found in certain patients with tertiary syphilis and paroxysmal hemoglobinuria. The disease process is therefore the result of the invisible union of a serum antibody with red cells in the cold, so that the sensitized red cells hemolyze on subsequent warming in the presence of complement (116)

Paroxysmal Nocturnal Hemoglobinuria. This curious disorder, originally described by Marchiafava and Nazari (118) and by Micheli (122), is a chronic acquired hemolytic anemia associated with intravascular hemolysis of the red cells, especially during sleep Although the paroxysms of severe hemolysis may be few and spaced far apart, there is a chronic low-grade hemolysis and perpetual hemosiderinuria (increased indirect bilirubin in the urine). A few transfusion survival studies have demonstrated that normal red

pear normal when examined on the smear (there are no spherocytes) and by the hypotonic fragility test, but they have been found to be abnormally fragile when placed in a mildly acid substrate. It has

vestigated We have seen such bodies following splenectomy with- the hypochromic red cells of a man who had a familial spleno- egalic hemolytic anemia, as well as in a number of patients fol- wing splenectomy for supposed splenic pancytopenia. McFadzean id Davis (120) found these bodies in seven cases of acquired idio- ıthic hemolytic anemia, and listed their characteristics as follows: ') They stained blue to purple with the Romanowsky stains. (2) hey stained positive with the Prussian blue reaction for iron. (3) hey did not stain with nuclear stains; hence they were not nuclear agments. (4) They tended to occur in deformed cells, cells which ere hypochromic, and with only a rim of hemoglobin (5) They :curred only in red cells and in cells of the reticulo-endothelial 'stem, including monocytes. (6) In normoblasts, they were present ıly when hemoglobin appeared in the cytoplasm

The ... 'haracteristically more numer- ıs a ... idiopathic hemolytic anemia ıan ... ıumber of *siderocytes in the* arrow was identical before and after splenectomy in these cases ıe difference in their number in the blood was probably due to ıeir removal by the spleen. That is, such cells were preferentially able to destruction by the spleen, giving rise to a hemolytic nemia; or the spleen inhibited their delivery to the blood. Sidero- ytes occurred, but were rare, in familial spherocytosis, in pernicious nemia, in the anemia of chronic infection, and in a few other ematologic disorders They were most frequent in certain acquired emolytic anemias of an idiopathic type, and were especially in- reased in both marrow and blood during hemolytic crises.

It is likely that siderocytes, at least in certain cases, are the eflection of an abnormal form of erythropoiesis which gives rise ɔ a deformed, hypochromic red cell with inclusions of nonhemo- lobinous iron When the defect is inherited, a familial siderocytic nemia may occur More often, apparently, the disturbance is ac- uired, and one subdivision of acquired idiopathic hemolytic anemia s siderocytic anemia This form of red cell abnormality will bear nuch further study and elucidation.

Symptomatic Hemolytic Anemia. A number of cases of ac- uired hemolytic anemia is found to be associated with a more undamental, underlying disease. Various examples of hemolytic nemia (47) in patients with carcinoma, sarcoma, Hodgkin's disease,

Role of Clot. Jordan found, in addition, that the phenomenon of clotting was necessary for sensitization of the cells *in vitro.* When cells of such patients were obtained from unclotted blood, they showed no hemolysis in the presence of complement and heat. When the cells were obtained from clotted blood, however, complement plus heat caused prompt hemolysis. Subsequent studies suggested that this effect was due to a reduction in complement by the anticoagulant material, rather than to a possible effect on the red cells or on the antibody affixed to them

Despite numerous studies, the cause and fundamental hemolytic mechanism for the disorder remain obscure The changes in the pH of the blood during sleep, although in the direction of increased acidity, are of such small caliber as to suggest that the *in vitro* increase in acid fragility of the cells is perhaps unrelated to the mechanism of their destruction *in vivo.* There is little evidence that the posture of the patient affects the hemolysis; in some cases, perhaps, hemolysis may be prevented by sleep in an erect position, but in others no such phenomenon occurs.

Treatment has no effect on the disorder. Alkali tends to prevent hemolysis for a while, but ultimately it recurs, and becomes exaggerated on cessation of the therapy Splenectomy is useless, and blood transfusions are necessary for symptomatic relief

"Siderocytic" Hemolytic Anemia. Grüneberg (82–84), in 1940 and the following years, described the occurrence, as an inherited abnormality in certain mice, [illegible]
positive granules within their [illegible]

[illegible]

nowsky stains, that they were positive with the ferrocyanide reaction (i e, the iron was not in the form of hemoglobin), and that they became prominent and abundant after splenectomy Identical bodies were found in the reticulo-endothelial cells of the spleen and elsewhere in the body, and similar bodies were found in five other patients after splenectomy and in various forms of hemolytic anemia

The significance of these bodies ("Pappenheimer bodies") and of the cells which contain them ("siderocytes") has recently been

it excessively liable to the normal forces of destruction, but to a more or less specific overactivity of the spleen, whereby its normal function of destroying effete red cells becomes converted and exaggerated into widespread destruction of normal red cells. It is characteristic of these cases that the hemolytic anemia is merely one of several effects of the spleen on the cells of the blood, so that leukopenia and thrombocytopenia are also often present. Since 1899, such cases have been described under various designations—aleukia splenica, splenic anemia, splenic neutropenia, hypersplenism (73, 93,100). Doan and co-workers (192,193) believe that the pancytopenia in these cases is due to sequestration and phagocytosis of granulocytes, erythrocytes, and platelets within the reticulo-endothelial elements of the spleen (phagocytic theory). Dameshek and his group have maintained that the spleen, in addition to its erythrolytic function, is also an endocrine organ whose function is to regulate the production and delivery of granulocytes, red cells, and platelets from the bone marrow (35,36). According to this theory, various forms of hypersplenism (neutropenia, anemia, thrombocytopenia, pancytopenia) result, depending upon which of the marrow cells are affected. In the particular variety of hypersplenism in which there is at the same time excessive destruction of large numbers of erythrocytes and inhibition of delivery of granulocytes and platelets from the marrow, hypersplenic hemolytic anemia occurs. Both groups of workers have demonstrated that removal of the spleen in these cases almost always leads to complete cure of the hematologic and clinical disorders.

These cases may be divided into two groups (36): (*1*) idiopathic hypersplenic hemolytic anemia, in which the enlargement of the spleen is of unknown origin, and in which the enlarged spleen is [illegible]

tomatic" hemolytic anemia in association with Hodgkin's disease, leukemia, Boeck's sarcoid (25), and other conditions in which the spleen, already enlarged by the underlying disease, becomes physiologically hyperactive To this group also belong, in all probability, other cases of hemolytic anemia in association with splenomegaly, such as chronic malaria, chronic rheumatoid arthritis, and infectious mononucleosis.

leukemia, ovarian teratoma (183), and other disorders, have long been known. The term "symptomatic hemolytic anemia" has been suggested for this group of cases, to emphasize the fact that their occurrence is secondary to a more basic disease (164).

These patients characteristically present the picture of ordinary spherocytic hemolytic anemia, and it is only after extensive study that it may be possible to assign to them their title of "symptomatic" The patient has the usual features of an acute or subacute hemolytic process: pallor, slight jaundice, splenomegaly. Investigation fails to reveal a positive family history. Study of the blood smear shows spherocytosis and polychromatophilia With the knowledge that a hemolytic anemia may overlie a more serious fundamental disease, a search for leukemia, carcinoma, lymphoma, etc., may be successful. In other cases, however, no underlying disease can be discovered; and it is only at operation or autopsy that the "symptomatic" nature of the hemolytic process is recognized.

These cases are apparently due to some "toxic" influence exerted upon the circulating red cells by the organs involved by the basic disease. The occurrence of spherocytosis is in line with the suggestion of a toxic substance affecting the red cells. Circulating antibodies, however, have not been demonstrated in these cases It is likely that certain of these cases, notably those associated with enlargement of the spleen (leukemia, Hodgkin's disease) may be due to "hypersplenism" In other cases, however, there is no evidence of any such mechanism Of especial interest are several cases of hemolytic anemia associated with ovarian teratoma in which hemolysis did not subside after splenectomy, but only *after the removal of the neoplasm itself Since the teratomas did not contain* splenic tissue, and showed no evidence of phagocytosis or lysis of erythrocytes, a humoral or "toxic" effect on circulating erythrocytes must be postulated.

Symptomatic hemolytic processes, though relatively rare, should be considered in association with various types of malignant disease and in certain benign disorders, especially when hemolysis does not cease following splenectomy (47,169)

Hypersplenic Hemolytic Anemia

Finally, there is a group of cases in which hemolytic anemia seems to be due not to a defect or injury of the red cell which makes

13 Caffey, J.: Skeletal changes in chronic hemolytic anemias (erythroblastic anemia, sickle cell anemia, and chronic hemolytic icterus). Am. J. Roentgenol. *37*, 293, 1937.
14 Callender, S. T. E, Loutit, J F, and Jope, E M · Discussion on the life and death of the red blood corpuscle Proc Roy Soc. Med *39*, 755, 1946.
15 Callender, S T. E, and Nickel, J. F.: Survival of transfused sickle cells in normal subjects and of normal red blood cells in patients with sickle cell anemia. J. Lab. & Clin Med. *32*, 1397, 1947.
16 Callender, S T E., Powell, E O, and Witts, L. J. The life-span of red cell in man J Path & Bact *57*, 129, 1945
17 Castle, W B, and Daland, G A . Susceptibility of erythrocytes to hypotonic hemolysis as a function of discoidal form Am J. Physiol. *120*, 371, 1937
18 Castle, W. B, and Daland, G A : Susceptibility of mammalian erythrocytes to hemolysis with hypotonic solutions; a function of differences between discoidal volume and volume of a sphere of equal surface Arch Int. Med *60*, 949, 1937
19a Chauffard, M A, and Troisier, J · Anémie grave avec hémolysine dans la sérum, ictère hémolysinique. Semaine méd *28*, 94, 1908.
19b Chauffard, M A, and Vincent, C Hémoglobinurie hémolysinique avec ictère polycholie aigue. Semaine méd. *29*, 601, 1909
20. Conrad, E, and Schmidt, R E: Congenital hemolytic anemia—a case requiring early splenectomy Am J. Dis. Child *72*, 731, 1946
21 Cooley, T B Elliptocytosis and anemia Am. J Dis Child. *64*, 190, 1942
22 Cooley, T. B Severe type of hereditary anemia with elliptocytosis Interesting sequence of splenectomy. Am J M Sc *209*, 561, 1945
23 Coombs, R R A, Mourant, A E, and Race, R R A new test for the detection of weak and "incomplete" Rh agglutinins. Brit J Exper Path *24*, 255, 1945
24 Cooper, E L Familial acholuric jaundice associated with bone changes Ann Int Med *15*, 858, 1941
25 Crane, A R, and Zetlin A M Hemolytic anemia, hyperglobulinemia, and Boeck's sarcoid Ann Int Med *23*, 882, 1945
26 Creevy, C D, and Webb, E A A fatal hemolytic reaction following transurethral resection of the prostate gland A discussion of its prevention and treatment Surgery *22*, 56, 1947
27 Currie, J P · Acute haemolytic anaemia· Report of a case presenting hitherto unreported features Brit M J *2*, 8, 1944
28 Dacie, J V, and Firth, D Blood transfusion in nocturnal haemoglobinuria Brit M J *1*, 626, 1943
29 Dacie, J V, and Mollison, P L Survival of normal erythrocytes after transfusion to patients with familial haemolytic anaemia (acholuric jaundice) Lancet *1*, 550, 1943
30 Dameshek, W "Target cell" anemia, anerythroblastic type of Cooley's erythroblastic anemia Am J M Sc *200*, 445, 1940
31 Dameshek, W Familial hemolytic crisis, report of three cases occurring within ten days New England J Med *224*, 52, 1941

In the group of "primary" or idiopathic hypersplenic cases belongs a small group of individuals with this specific variety of acquired idiopathic hemolytic anemia. It is characteristic of these patients that the usual hematologic reaction to hemolysis—granulocytic leukocytosis and thrombocytosis, due to a pouring out of cells from the highly reactive marrow—does not occur, so that the patient shows hemolytic anemia in association with leukopenia, neutropenia, and thrombocytopenia, despite the hyperplasia of these elements in the marrow.

✓ Splenectomy in these cases is almost always followed by cure of the hemolytic anemia, although any fundamental disease present (in the secondary cases) is not affected (36).

References

1 Ashby, W Determination of length of life of transfused blood corpuscles in man J Exper. Med *29*, 267, 1919.

2. Belson, M. O . Rh factor in obstetrics Am J Obst & Gynec *52*, 106, 1946.

3 Bergenhem, B, and Fåhraeus, R Ueber spontane Hemolysinbildung im Blut unter besonderer Berucksichtigung der Physiologie der Milz Ztschr f d ges exper Med *97*, 555, 1936

4 Bessis, M, and Freixa, P. Études sur l'ictère hémolytique expérimental par injection et ingestion d'antisérum Rev hemat Paris *2*, 114, 1947.

5 Bloxsom, A Hemolytic disease of the newborn (erythroblastosis fetalis) Treatment by a single massive transfusion, with complete recovery Am J. Dis. Child *72*, 320, 1946

6 Bloxsom, A The Rh-positive factor in congenital hemolytic disease (erythroblastosis fetalis) South M J *40*, 829, 1947

7 Boorman, K E, Dodd, B E, and Loutit, J F Haemolytic icterus (acholuric jaundice), congenital and acquired Lancet *1*, 812, 1946

8 Boorman, K E, Dodd, B E, Loutit, J. F, and Mollison, P L Some results of transfusion of blood to recipients with "cold" agglutinins Brit M J *1*, 751, 1946

9 Boorman, K E, Dodd, B E, and Mollison, P L The incidence of haemolytic disease of the foetus ("erythroblastosis foetalis") in different families, the value of serological tests in diagnosis and prognosis J. Obst. & Gynaec Brit Emp *51*, 1, 1944

10 Brown, A Morphological changes in the red cells in relation to severe burns J Path & Bact *58*, 367, 1946

11 Brown, G. M, Hayward, O C, Powell, E O, and Witts, L J · The destruction of transfused erythrocytes in anaemia J Path & Bact *56*, 81, 1944

12 Buxton, B H, and McDuff, H C Erythroblastosis fetalis Rhode Island M J *28*, 333, 1945

54 Diamond, L. K., and Denton, R. L.: Rh agglutination in various media with particular reference to the value of albumin. J. Lab. & Clin. Med *30*, 821, 1945.

55. Diamond, L. K., and Denton, R. L. Treatment of erythroblastosis fetalis. J. A. M. A. *128*, 686, 1945.

56 Donath, J., and Landsteiner, K.: Ueber paroxysmale Hämoglobinurie München. med. Wchnschr. *51*, 1590, 1904

57. Drummond, R.: Symptomless incompatible transfusion and resultant changes in iso-agglutinin titre Brit M. J. *1*, 483, 1944.

58 Ebert, R. V., and Emerson, C. P., Jr. Clinical study of transfusion reactions; hemolytic effect of group-O blood and pooled plasma containing incompatible isoagglutinins J Clin Investigation *25*, 627, 1946.

59 Eppinger, H: Über schwerheilbare Fussgeschwüre bein hämolytischen Ikterus. Klin. Wchnschr *9*, 10, 1930

60 Estes, J. E, Farber, E M, and Stickney, J. M.: Ulcers of the leg in Mediterranean disease. Blood *3*, 302, 1948

61. Estiu, M., and Lenci, P.: Quoted in West-Watson, W. N, and Young, C. J (183).

62 Evans, R. S.: Acute hemolytic anemia with autoagglutination, case report Stanford M Bull *1*, 178, 1943

63 Evans, R. S: Chronic hemolytic anemia; observations of the effect of fat content of the diet and multiple red cell transfusions. Arch Int Med *77*, 544, 1946

64 Evans, R S, and Duane, R T Observations on the effect of irradiation in chronic acquired hemolytic anemia exhibiting hemolytic activity for transfused erythrocytes Blood *2*, 72, 1947

65 Evans, R S, Duane, R. T, and Behrendt, V Demonstration of antibodies in acquired hemolytic anemia with anti-human globulin serum Proc Soc Exper Biol & Med *64*, 372, 1947

66 Faber, H K, and Roth, B M: Mediterranean anemia (Cooley) in a Negro girl J Pediat *28*, 610, 1946

67 Farrar, G E, Jr, Burnett, W E, and Steigman, A J: Hemolysin anemia and hepatic degeneration cured by splenectomy. Am J M. Sc *200*, 164, 1940

68 Findlay, G M, Robertson, W M., and Zacharias, F J The incidence of sicklemia in West Africa Tr Roy Soc Trop Med & Hyg *40*, 83, 1946.

69 Finland, M, Peterson, O L, Allen, H E, Samper, B A, and Barnes, M W: Cold agglutinins II Cold isohemagglutinins in primary atypical pneumonia of unknown etiology with a note on the occurrence of hemolytic anemia in these cases J Clin Investigation *24*, 458, 1945

70 Forbes, G B Autohemagglutination and Raynaud's phenomenon. Brit M J *1*, 598, 1947

71 Fox, C L, Jr, and Ottenberg, R Acute hemolytic anemia from the sulfonamides. J Clin Investigation *20*, 593, 1941.

32 Dameshek, W : Familial Mediterranean target–oval cell syndromes. Am J M Sc *205*, 643, 1943
33 Dameshek, W.. The hemolytic crisis [Editorial] Blood *3*, 307, 1948
34 Dameshek, W Unpublished observations
35 Dameshek, W., and Estren, S : Hypersplenism· some preliminary observations Acta med Scandinav. Supp No 213, 106, 1948
36 Dameshek, W, and Estren, S The Spleen and Hypersplenism New York, Grune & Stratton, 1948.
37 Dameshek, W, Greenwalt, T J, and Tat, R J · Erythroblastosis foetalis (acute hemolytic anemia of the newborn), preliminary report Am J Dis Child *65*, 571, 1943
38 Dameshek, W, Greenwalt, T J, Tat, R J, and Dreyfus, C.. Hemolytic Syndromes Boston, Bailey Press, 1942
39 Dameshek, W, and Henstell, H H Unpublished observations
40 Dameshek, W, and Miller, E B Pathogenetic mechanisms in hemolytic anemias Arch Int Med *72*, 1, 1943
41 Dameshek, W, and Schwartz, S O The presence of hemolysins in acute hemolytic anemia New England J Med *218*, 75, 1938
42 Dameshek, W, and Schwartz, S O Hemolysins as the cause of clinical and experimental hemolytic anemias with particular reference to the nature of spherocytosis and increased fragility Am J. M Sc *196*, 769, 1938
43 Dameshek, W, and Schwartz, S O Acute hemolytic anemia (acquired hemolytic icterus, acute type) Medicine *19*, 231, 1940
43a Quoted in (43), references 8,68,121,126,264,300,334,364,369; also (43)
43b Quoted in (43), references 50,214,300,343. also (43)
43c Quoted in (43), references 47,50,85,130,131,132,223,300,331,332, also (43)
43d Quoted in (43), references 15,125,214, also (43)
44 Danis, P G, Eto, J K, and Sennott, J S The use of Rh-positive blood cells in the treatment of erythroblastosis fetalis J Pediat *28*, 457, 1946
45 Darrow, R R The treatment of erythroblastosis fetalis J A M A *127*, 1146, 1945
46 David, J K, Jr, and Minot, A S Hemolytic anemia in infancy report of case with demonstration of hemolytic activity of serum Am J Dis Child *68*, 327, 1944
47 Davis, L J Symptomatic haemolytic anaemia A report of four cases Edinburgh M J *51*, 70, 1944
48 Dawson, B E Haemolytic icterus Brit M J *1*, 921, 1931
49 Dedichen, H G Epidemic occurrence of anemic crises in hemolytic jaundice Brit M J (Epitome) *1*, 73, 1937
50 Dekkers, H J N The fate of the transfused red blood cells Acta med Scandinav *99*, 587, 1939
51 Diamond, L K The clinical importance of the Rh blood type New England J Med *232*, 447, 1945
52 Diamond, L K. Erythroblastosis foetalis or haemolytic disease of the newborn Proc Roy Soc Med *40*, 546, 1947
53 Diamond, L K : Quoted in Levine, P. (110).

90. Harrison, H. E., Bunting, H., Ordway, N. K., and Albrenk, W. S.: The pathogenesis of the renal injury produced in the dog by hemoglobin or methemoglobin. J. Exper. Med *86*, 339, 1947.
91 Hawksley, J C: Acholuric family jaundice in an infant. Proc. Roy. Soc. Med *27*, 912, 1934
92 Hegglin, R, and Maier, C.: The "heat resistance" of erythrocytes A specific test for the recognition of Marchiafava's anemia. Am. J. M. Sc *207*, 624, 1944.
93 Heilmeyer, L · Die hämolytische Hypersplenie. Beitrag zur Frage des erworbenen hämolytischen Ikterus Deutsches Arch. f. klin. Med *178*, 89, 1935
94 Henderson, A B, and Thornell, H. E: Observations on the effect of lowered oxygen tension on sicklemia and sickle cell anemia among military flying personnel J. Lab & Clin. Med *31*, 769, 1946.
95 Hertzog, A J · Problems encountered in explaining certain cases of erythroblastosis fetalis on the Rh theory. Minnesota Med *26*, 1057, 1943
96 Hill, J M, and Haberman, S: Demonstration of Rh antibodies in newborn and further evidence of pathogenesis of erythroblastosis J. Lab. & Clin Med *31*, 1053, 1946
97. Hill, J. M, and Haberman, S: Hemolytic Rh immune globulins; evidence for a possible third order of antibodies incapable of agglutination or blocking Blood Special issue No. 2, 80, 1948
98 Horne, E O Congenital hemolytic icterus Surgical treatment of complications with a report of two cases Am J Surg *65*, 56, 1944
99 Horne, J L, Lederer, H, Kirkpatrick, H J. R, and Leys, D. G.: Familial crisis in congenital haemolytic disease Lancet *2*, 1, 1945
100 Isaac, S · Ueber Anaemia splenica Berl klin Wchnschr *42*, 1978, 1912
101 Johnson, V, Freeman, L W, and Longini, J. Erythrocyte damage by lipemic serum in normal man and in pernicious anemia J. A M. A. *124*, 1250, 1944
102 Jope, E M Disappearance of sulphemoglobin from blood of TNT workers in relation to dynamics of red cell destruction. Brit J. Indust Med *3*, 136, 1946.
103 Jordan, F L J Études sur l'hémoglobinurie. Acta Med Scandinav *95*, 319, 1938
104 Koteen, P, and Brooks, N Establishment of a transfusion clinic Treatment of Mediterranean anemia in the outpatient department J Pediat *28*, 697, 1946.
105 Kracke, R R, and Hoffman, B J. Chronic hemolytic anemia with autoagglutination and hyperglobulinemia; report of fatal case Ann Int Med *19*, 673, 1943
106 Lalich, J. J · The influence of injections of homologous hemoglobin on the kidneys of normal and dehydrated animals J Exper Med *86*, 153, 1947
107 LeWald, L T · Roentgen evidence of osseous manifestations in sickle cell (drepanocytic) anemia and Mediterranean (erythroblastic) anemia. Radiology *18*, 792, 1932

72 Foy, H, and Kondi, A.: Lyso-lecithin fragility in blackwater fever and haemolytic jaundice Trans Roy Soc Trop Med & Hyg *37*, 1, 1943
73 Frank, E · Aleukia splenica Die splenogene Leukomyelotoxikose. Aleukia haemorrhagica III. Berl klin Wchnschr. *53*, 555, 1916
74 Freeman, L W., Loewy, A, and Johnson, A.. Physiologic icterus of the newborn Federation Proc *5*, 30, 1946
75 Gansslen, M.. Der hamolytische Ikterus. Deutsches Arch f. klin Med. *140*, 210, 1922.
76 Gilligan, D R, and Blumgart, H. L : March hemoglobinuria; studies of the clinical characteristics, blood metabolism and mechanism; with observations on three new cases, and review of literature Medicine *20*, 341, 1941.
77. Ginsberg, H S · Acute hemolytic anemia in primary atypical pneumonia associated with high titer of cold agglutinins, report of a case New England J Med *234*, 826, 1946
78 Govan, C D : Erythroblastic anemia of Cooley; observations on the effect of splenectomy performed on identical twins J Pediat *29*, 504, 1946
79 Greenblatt, K J, Cohn, T D, and Deutsch, H L · Mediterranean target-oval cell syndome in an adult Chinese male; report of a case Ann Int Med *24*, 259, 1946
80 Greenwald, L, Spielholz, J B, and Litwins, J Sickling trait in a white adult associated with hemolytic anemia, endocarditis and malignancy Am J. M Sc *206*, 158, 1943
81 Grinnan, A G Roentgenologic bone changes in sickle cell and erythroblastic anemia Am J Roentgenol *34*, 297, 1935
82 Gruneberg, H Siderocytes A new kind of erythrocytes Nature *148*, 114, 1941
83 Grüneberg, H The Genetics of the Mouse London, Cambridge Univ Press, 1943
84 Gruneberg, H Inherited disorders of the blood in rodents Proc Roy Soc Med *40*, 191, 1947
85 Haden, R L The mechanism of increased fragility of the red blood cells in congenital hemolytic jaundice Am J M Sc *188*, 411, 1934
86 Ham, T H Studies on destruction of red blood cells, chronic hemolytic anemia with paroxysmal nocturnal hemoglobinuria investigation of mechanism of hemolysis, with observations on 5 cases Arch Int Med *64*, 1271, 1939
87 Ham, T H, and Castle, W B Studies on destruction of red blood cells Proc Am Philos Soc *82*, 411, 1940
88 Ham, T. H, and Castle, W B Relation of increased hypotonic fragility and of erythrostasis to the mechanism of hemolysis in certain anemias Tr. A Am. Physicians *55*, 127, 1940
89 Ham, T H, and Dingle, J H Studies on destruction of red blood cells, chronic hemolytic anemia with paroxysmal nocturnal hemoglobinuria Certain immunologic aspects of hemolytic mechanism with special reference to serum complement J Clin Investigation *18*, 657, 1939

90. Harrison, H E, Bunting, H, Ordway, N. K, and Albrenk, W. S.: The pathogenesis of the renal injury produced in the dog by hemoglobin or methemoglobin J Exper. Med. *86*, 339, 1947.
91. Hawksley, J C: Acholuric family jaundice in an infant Proc Roy. Soc Med. *27*, 942, 1934.
92 Hegglin, R, and Maier, C: The "heat resistance" of erythrocytes A specific test for the recognition of Marchiafava's anemia Am. J. M Sc. *207*, 624, 1944
93. Heilmeyer, L: Die hämolytische Hyperspleme. Beitrag zur Frage des erworbenen hämolytischen Ikterus Deutsches Arch. f klin. Med *178*, 89, 1935
94 Henderson, A. B, and Thornell, H E: Observations on the effect of lowered oxygen tension on sicklemia and sickle cell anemia among military flying personnel J Lab & Clin Med. *31*, 769, 1946
95 Hertzog, A J Problems encountered in explaining certain cases of erythroblastosis fetalis on the Rh theory Minnesota Med *26*, 1057, 1943
96 Hill, J M, and Haberman, S: Demonstration of Rh antibodies in newborn and further evidence of pathogenesis of erythroblastosis J Lab & Clin. Med *31*, 1053, 1946
97 Hill, J M, and Haberman, S: Hemolytic Rh immune globulins; evidence for a possible third order of antibodies incapable of agglutination or blocking Blood Special issue No 2, 80, 1948
98 Horne, E. O Congenital hemolytic icterus Surgical treatment of complications with a report of two cases Am J Surg *65*, 56, 1944
99 Horne, J L, Lederer, H, Kirkpatrick, H J R, and Leys, D. G Familial crisis in congenital haemolytic disease. Lancet *2*, 1, 1945
100 Isaac, S: Ueber Anaemia splenica Berl klin Wchnschr. *42*, 1978, 1912
101 Johnson, V, Freeman, L W, and Longini, J: Erythrocyte damage by lipemic serum in normal man and in pernicious anemia J. A M. A *124*, 1250, 1944
102 Jope, E M Disappearance of sulphemoglobin from blood of TNT workers in relation to dynamics of red cell destruction Brit. J Indust Med *3*, 136, 1946
103 Jordan, F L J Études sur l'hémoglobinurie Acta Med Scandinav. *95*, 319, 1938
104 Koteen, P, and Brooks, N Establishment of a transfusion clinic Treatment of Mediterranean anemia in the outpatient department J Pediat *28*, 697, 1946
105 Kracke, R R, and Hoffman, B J Chronic hemolytic anemia with autoagglutination and hyperglobulinemia, report of fatal case Ann Int Med *19*, 673, 1943
106 Lalich, J J The influence of injections of homologous hemoglobin on the kidneys of normal and dehydrated animals J Exper. Med *86*, 153, 1947
107 LeWald, L T Roentgen evidence of osseous manifestations in sickle cell (drepanocytic) anemia and Mediterranean (erythroblastic) anemia Radiology *18*, 792, 1932

108 Leitner, S J.. Weitere Untersuchungen uber die familiare Elliptozytose der Erythrozten Elliptozytose und hamolytische Anamie. Helvet Med Acta *10*, 585, 1943.

109 Levine, P.: Recent developments in iso-immunization by Rh factor Am J Obst. & Gynec *49*, 810, 1945

110 Levine, P.. A survey of the significance of the Rh factor. Blood Special issue No. 2, 3, 1948.

111 Levine, P, and Waller, R. K.. Erythroblastosis foetalis in the first-born; prevention of its most severe forms Blood *1*, 143, 1946

112 Loutit, J F., and Mollison, P. L Haemolytic icterus (acholuric jaundice), congenital and acquired. J Path & Bact *58*, 711, 1946

113 Lowe, R C. A study of hemoglobin metabolism and hematology in a case of congenital hemolytic jaundice during (A) clinical crisis, (B) repeated transfusions, and (C) before and after splenectomy. Am. J M. Sc *206*, 347, 1943

114 Lubinski, H., and Goldbloom, A Acute hemolytic anemia associated with autoagglutination with a thermal amplitude of 0 to 37 C Am J. Dis Child *72*, 325, 1946

115 Lyngar, E. Nord. med *14*, 1246, 1942

116 Mackenzie, G. M.. Paroxysmal hemoglobinuria, a review Medicine *8*, 159, 1929

117 Maegraith, B, Findlay, G M, and Martin, N H Mechanism of lysis of red blood cells Nature *151*, 252, 1943

117a Maegraith, B G, Martin, N. H, and Findlay, G M The mechanism of red blood cell destruction Brit J Exper Path *24*, 58, 1943

118 Marchiafava, E, and Nazari, A Nuova contributa allo studio degli itteri chronici emolitici Policlinico (sez med) *18*, 241, 1911

119 McCall, A. J, Race, R R, and Taylor, G L Rhesus antibody in Rh-positive mother causing haemolytic disease of newborn. Lancet *1*, 214, 1944

120 McFadzean, A J S, and Davis, L J · Iron-staining erythrocytic inclusions with especial reference to acquired haemolytic anaemia Glasgow M J *28*, 237, 1947.

121 Merskey, C, and Baskind, E Chronic hemolytic icterus resembling acholuric jaundice occurring in an African native South African M J *20*, 230, 1946

122 Micheli, F Anemia splenomegalia emolitica con emoglobinuria emosiderinuria tipo Marchiafava Haematologica *12*, 101, 1931

123 Miller, E B, Singer, K, and Dameshek, W : Use of daily fecal output of urobilinogen and hemolytic index in measurement of hemolysis. Arch Int Med *70*, 722, 1942

124 Minot, G R, and Castle, W B (editors) In Yearbook of General Medicine 1944, p 353 Chicago, Year Book Publishers, 1944

125 Mogensen, E The Size of the Red Blood Cells Copenhagen, Munksgaard, 1938

126 Mollison, P L · The survival of transfused erythrocytes in haemolytic disease of the newborn Arch. Dis Childhood *18*, 161, 1943.

126a Mollison, P. L. Quoted in Loutit, J F., and Mollison, P. L (112).
127. Mollison, P. L, and Young, I M : *In vivo* survival in the human subject of transfused erythrocytes after storage in various preservative solutions. Quart. J Exper Physiol *31*, 359, 1942.
128. Morgan, A. D, and Lumb, G : Fatal use of a dangerous universal donor. Lancet *2*, 866, 1946
129. Morrison, M., Samwick, A A, and Landsberg, E : Sickle cell anemia in the white race; report of two cases with diagnosis by splenic puncture Am. J Dis Child *64*, 881, 1942
130. Murphy, R. C, and Shapiro, S.: Sickle cell disease I Observations on behavior of erythrocytes in sickle cell disease Arch Int. Med. *74*, 28, 1944.
131 Murphy, R. C, and Shapiro, S. The pathology of sickle cell disease Ann. Int Med *23*, 376, 1945
132 Murray-Lyon, R M. Familial acholuric jaundice simulating Lederer's anaemia Brit M. J *1*, 50, 1935
133 Naegeli, O. Blutkrankheiten und Blutdiagnostik, 3d ed., p 308. Berlin, de Gruyter, 1919
134. Napier, L E., Sen Gupta, P C., Bhaduri, N. V., and Sekar, C. C.: Studies in haemolysis Indian J M Research *31*, 75, 1943
135 Neber, J, and Dameshek, W The improved demonstration of circulating antibodies in hemolytic anemia by the use of a bovine albumin medium Blood *2*, 371, 1947
135a Neber, J, and Dameshek, W Unpublished observations
136 Neel, J. V, and Valentine, W N The frequency of thalassemia. Am J M Sc *209*, 568, 1945
137 Ogden, M A · Sickle cell anemia in white race, with report of cases in 2 families Arch Int Med. *71*, 164, 1943
138 Owren, P Congenital hemolytic jaundice, the pathogenesis of the "hemolytic crisis" Blood *3*, 231, 1948
139 Pappenheimer, A M, Thompson, W P, Parker, D D, and Smith, K. E Anaemia associated with unidentified erythrocytic inclusions after splenectomy Quart J Med *54*, 75, 1945
140 Polayes, S H Erythroblastosis fetalis in mothers with Rh positive blood, a report of six cases, with comment on isoimmunization with the "A" and "B" agglutinogens Am J Dis Child *69*, 99, 1945
141 Polayes, S H, and Ohlbaum, C Erythroblastosis foetalis unrelated to Rh factor Am J Clin Path *15*, 467, 1945
142 Ponder, E Permeability of red cell membrane after hypotonic hemolysis Proc Soc Exp Biol & Med *33*, 630, 1936
143 Ponder, E The spherical form of the mammalian erythrocyte. III Changes in surface area in discs and spheres J Exper. Biol. *14*, 267, 1937
144 Ponder, E Red cell cytochemistry and architecture. Ann New York Acad Sc *48*, 579, 1947
145 Race, R R · The Rh blood groups Schweiz med Wchnschr *76*, 921, 1946

146 Race, R R The Rh genotypes and Fisher's theory. Blood Special issue No 2, 27, 1948
147 Reisner, E H, Jr, and Kalkstein, M. Autohemolysinic anemia with autoagglutination Improvement after splenectomy. Am J. M. Sc *203*, 313, 1942.
148 Ross, J. F, and Paegel, B. L · Acute hemolytic anemia and hemoglobinuria following sulfadiazine medication Blood *1*, 189, 1946
149 Rous, P : The destruction of the red blood corpuscles in health and disease Physiol Rev *3*, 75, 1923
150 Rundles, R. W, and Falls, H F Hereditary (?sex-linked) anemia Am J M. Sc *211*, 641, 1946
151 Saracoglu, K. Cooleysche Anamie in der Turkei Wien med. Wchnschr *93*, 217, 1943
152. Scherer, J H, and Cecil, R C Congenital hemolytic anemia in Negro J Lab & Clin Med *30*, *244*, *1945*
153 Schieber, C Target-cell anaemia, two cases in Bucharan Jews Lancet *2*, 851, 1945
154 Schiødt, E : On the duration of life of the red blood corpuscles Acta med Scandinav *95*, *49*, *1938*
155 Scott, A M : Acholuric jaundice, the serial onset of acute blood crises in entire family Lancet *2*, 872, 1935
156 Shemin, D, and Rittenberg, D The life span of the human blood cell J Biol Chem *166*, 627, 1946
157 Shemin, D, and Rittenberg, D The biological utilization of glycine for the synthesis of the protoporphyrin of hemoglobin J. Biol Chem *166*, 621, 1946
158 Shen, S C, Castle, W B, and Fleming, E M Experimental and clinical observations on increased mechanical fragility of erythrocytes Science *100*, 387, 1944
159 Shen, S C, Ham, T H, and Fleming, E M · Studies in destruction of red blood cells III Mechanism and complications of hemoglobinuria in patients with thermal burns, spherocytosis and increased osmotic fragility of red blood cells New England J. Med *229*, 701, 1943
160 Singer, K The lysolecithin fragility test Am J M Sc *199*, 466, 1940
161 Singer, K · Lysolecithin and hemolytic anemia The significance of lysolecithin production in the differentiation of circulating and stagnant blood J Clin Investigation *20*, 153, 1941.
162 Singer, K Problems of erythrocyte disintegration with particular reference to the life span of the red cell J Lab & Clin Med *30*, 784, 1945
163 Singer, K., and Rubin, S Rapid test for the demonstration of sickle cells and its clinical significance J A M A *136*, 1021, 1948
164 Singer, K., and Dameshek, W Symptomatic hemolytic anemia Ann Int. Med *15*, 544, 1941
165 Singer, K, and Weisz, L The *life cycle of the erythrocyte after* splenectomy and the problems of splenic hemolysis and target cell formation Am. J M Sc *210*, 301, 1945

166 Smith, C. H : Familial blood studies in cases of Mediterranean (Cooley's) anemia, diagnosis of the trait, or mild form of the disease. Am. J Dis Child *65*, 681, 1943
167. Stacey, R. S. Recurrent fatal hemolytic anemia associated with gross liver damage and splenomegaly. Am J. M Sc *212*, 586, 1946.
168 Stats, D · Cold hemagglutination and cold hemolysis, the hemolysis produced by shaking cold agglutinated erythrocytes. J. Clin. Investigation *24*, 33, 1945.
169 Stats, D., Rosenthal, N , and Wasserman, L R : Hemolytic anemia associated with malignant diseases Am J Clin. Path. *17*, 585, 1947.
170 Stats, D , and Wasserman, L. R.· Cold hemagglutination; an interpretive review. Medicine *22*, 363, 1943
171 Stiles, M. H , Manlove, C H , and Dangerfield, C. D : Mediterranean anemia, report of a case in a Negro. Northwest Med. *45*, 23, 1946.
172 Stragnell, R , and Smith, K. E : Congenital hemolytic jaundice in a Negro family. Am J. Med. *1*, 53, 1946
173 Stransky, E , and Regala, A C . New type of familial congenital chronic hemolytic anemia Am J Dis Child *71*, 492, 1946
174. Strauss, M B, Daland, G A, and Fox, H. J : Familial microcytic anemia; observations on 6 cases of a blood disorder in an Italian family Am J M Sc *201*, 30, 1941
175 Taylor, E S · Chronic ulcer of the leg associated with congenital hemolytic jaundice J A M A *112*, 1574, 1939
176. Tisdall, L H , Garland, D M , Szanto, P B , Hand, A M , and Bonnett, J C. The effects of the transfusion of group O blood of high isoagglutinin titer into recipients of other blood groups Am. J Path *16*, 193, 1946
177 Tsai, C , Lee, J , and Wu, C H The role of splenic action in altering erythrocytic fragility Chinese J Physiol *15*, 165, 1940
178 Valentine, W. N , and Neel, J V Hematologic and genetic study of the transmission of thalassemia (Cooley's anemia; Mediterranean anemia) Arch Int Med *74*, 185, 1944
179 Valentine, W N , and Neel, J V The artificial production and significance of target cells with special reference to their occurrence in thalassemia (Cooley's erythroblastic anemia). Am. J M Sc *209*, 741, 1945
180 Vogel, P · Quoted in Levine (110)
181 Wallerstein, H Substitution transfusion A new treatment for severe erythroblastosis fetalis Am J Dis Child *73*, 19, 1947.
182 Watson, C J Some newer concepts of the natural derivatives of hemoglobin Blood *1*, 99, 1946
183 West-Watson, W N , and Young, C J Failed splenectomy in acholuric jaundice, and the relation of toxaemia to the haemolytic crises Brit M J *1*, 1305, 1938
184 Whittle, C H , Lyell, A , and Gatman, M Raynaud's phenomenon, with paroxysmal haemoglobinuria, caused by cold haemagglutination Proc Roy Soc Med *40*, 500, 1947

185 Wiener, A. S Longevity of the erythrocyte. J. A. M A. *102*, 1779, 1934
186 Wiener, A S · Hemolytic transfusion reactions I. Diagnosis, with special reference to the method of differential agglutination. Am J. Clin Path. *12*, 189, 1942
187. Wiener, A S . Problem of the Rhesus antigen in medicine. In: Advances in Internal Medicine, Vol II, p. 439. New York, Interscience, 1947.
188 Wiener, A. S , and Gordon, E. B : Studies on the conglutination test in erythroblastosis fetalis. J. Lab & Clin. Med. *33*, 181, 1948.
188a Wiener, A S , and Hurst, J G.: A new test (direct test) for Rh blocking antibodies. Exper. Med & Surg *5*, 281, 1947.
189 Wiener, A S, Wexler, I B, and Grundfast, T. H.: Therapy of erythroblastosis fetalis with exchange transfusion Bull New York Acad. Med *23*, 207, 1947
190. Willenegger, H · Das Schicksal transfundierter Erythrocyten. Helvet. Med Acta *9*, 15, 1942.
191 Wintrobe, M M, Matthews, E, Pollack, R, and Dobyns, B M.: A familial hemopoietic disorder in Italian adolescents and adults. J. A. M A *114*, 1530, 1940
192 Wiseman, B K, and Doan, C A : A newly recognized granulopenic syndrome caused by excessive splenic leukolysis and successfully treated by splenectomy J Clin Investigation *18*, 473, 1939.
193 Wiseman, B K, and Doan, C A · Primary splenic neutropenia, a newly recognized syndrome, closely related to congenital hemolytic icterus and essential thrombocytopenic purpura Ann Int Med *16*, 1097, 1942
194 Witts, L J The paroxysmal haemoglobinurias Lancet *2*, 115, 1936
195 Woofter, A C, Dick, W S, and Bierring, W L · Sickle cell anemia in white patients with ulcers of the ankles, report of two cases Arch. Int Med *76*, 230, 1945
196 Young, L E, and Lawrence, J S Atypical hemolytic anemia; observations with particular reference to the use of transfusions in the study of hemolytic mechanisms Arch Int Med *77*, 151, 1946.
197. Yuile, C L, Gold, M A, and Hinds, E G Hemoglobin precipitation in renal tubules, a study of its causes and effects J Exper Med. *82*, 361, 1945
198 Zoutendyk, A The incidence of the "dangerous" group O donor Results of the titration of one thousand consecutive sera South African M J. *21*, 438, 1947

Host, Drug, and Parasite Factors That Modify the Therapeutic Activity of Penicillin

HARRY EAGLE

U. S Public Health Service, and The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland

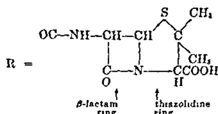
Introduction

Although the natural defense mechanisms of the body, whether humoral or cellular, may contribute to the therapeutic activity of penicillin, its direct bactericidal action is of major and perhaps primary importance It follows that the concentration of penicillin which is most effectively bactericidal, and the rate at which the particular organism is killed at that effective level, are important therapeutic considerations. It is however equally clear that the susceptibility of the invading organism to penicillin, and the provision of an effective level in the circulating blood, do not alone determine the outcome. The accessibility of the infectious focus to penicillin, the age of the infection, the number of organisms, and their rate of multiplication, are all of major significance. The therapeutic administration of penicillin is further complicated by the fact that there are several molecular species of penicillin differing in their pharmacologic properties and therapeutic activity, and several types of pharmaceutical preparation (e g, aqueous solutions, suspension in oil and beeswax, tablets for peroral administration, micronized penicillin for inhalation) which provide blood and tissue levels of widely varying magnitude and duration. Further, the rapid urinary excretion of the drug makes it difficult to provide a therapeutically effective concentration for prolonged periods by means of a single injection These must therefore be repeated at intervals and at dosages which will vary with the susceptibility of the organism and the vehicle in which the penicillin is administered. Due regard must be paid to the fact that if the injections are given too infrequently or in too small a dosage, the blood and tissue levels

may fall below those which are bactericidal for a sufficient period of time to permit the interim multiplication of organisms, and thus prejudice the outcome of treatment.

TABLE I

Naturally Occurring Species of Penicillin, All Possessing a Common Nuclear Structure (R)



Penicillin species	Prosthetic group which characterizes individual species of penicillin	
	Prosthetic group	Formula
F	Δ^2-Pentenyl-	$CH_3—CH_2—CH{=}CH_2—CH_2—R$
G	Benzyl-	C_6H_5—$CH_2—R$
X	*p*-Hydroxybenzyl-	OH—C_6H_4—$CH_2—R$
K	*n*-Heptyl-	$CH_3—CH_2—CH_2—CH_2—CH_2—CH_2—CH_2—R$
Dihydro F	*n*-Amyl	$CH_3—CH_2—CH_2—CH_2—CH_2—R$

Some of these factors are considered in the following discussion So little is as yet known as to the mode of action of penicillin, or the degree to which its therapeutic efficacy involves the participation of the cellular or humoral defense mechanisms of the host, that these will not be considered in the present chapter. Their final elucidation may cause a material revision in conclusions based primarily on the pharmacologic properties of the drug, its direct bactericidal action *in vitro*, and the available data as to its therapeutic activity in experimental and human infections

Multiplicity of Penicillins, and Their Varying Bactericidal Activity

Five penicillin species (F, dihydro-F, G, K, and X) have already been isolated and identified (1), and there is reason to suspect that there may be other "natural" penicillins not yet identified In addition, a number of chemically modified penicillins have been pro-

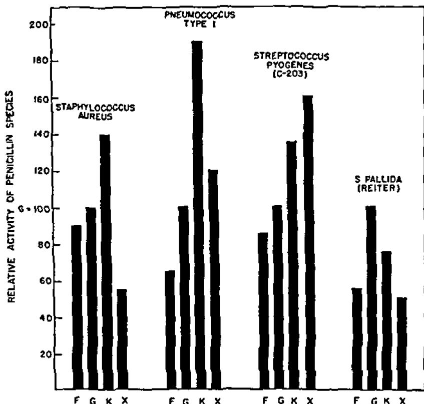


Fig 1. Varying bactericidal activity *in vitro* of penicillins F, G, K, and X (6,10)

duced by introducing into the nutrient medium appropriate chemical precursors which are not present in the mediums ordinarily used, and which the mold can use in building up a slightly different penicillin molecule.

The penicillins so far identified all possess a common nuclear structure, and differ in the nature of a side group attached to that common nucleus (Table I). The important practical consideration is that the several penicillins differ significantly and unpredictably in their activity against individual bacteria (2–12), and differ also in their pharmacologic behavior *in vivo* (page 120) (7,8,10,13–17). The twofold to threefold differences in the relative bactericidal action of penicillins F, G, K, and X against single strains of strepto-

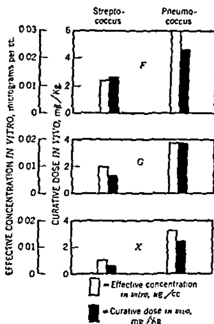


Fig 2 Correlation between susceptibility of streptococci and pneumococci to penicillins F, G, K, and X *in vitro*, and their curative dose *in vivo* (10).

cocci, staphylococci, pneumococci, and cultured spirochetes *in vitro* are shown in Figure 1 One would anticipate that, other things being equal, a greater bactericidal activity *in vitro* would be reflected by a greater therapeutic activity *in vivo* This has been found to be the case with penicillins F, G, and X in the treatment of experimental mouse infections with pneumococci and streptococci (10) (Fig. 2) Penicillin X, which was the most effective against a given organism

in vitro, was also the most effective *in vivo*. Similarly, streptococci, which were more susceptible than pneumococci to all three penicillins *in vitro*, were also more easily killed *in vivo*. As will be presently discussed, this generalization requires qualification in many respects; but by and large the direct bactericidal action of penicillin is an important factor in determining its therapeutic activity. The wide differences in the activity of penicillins F, G, and X in the treatment of experimental syphilis (Table IV) probably reflect corresponding differences in their direct treponemicidal activity.

The implications of these differences in the bactericidal activity of the several species of penicillin with respect to their clinical use are discussed later.

Penicillin Susceptibility of Individual Bacterial Species and Strains

Some organisms are killed by as little as 0 004 μg. of penicillin per cubic centimeter; others grow luxuriantly in that concentration, but are killed at 0 1 μg per cubic centimeter, for example; still others show no evidence of susceptibility save at concentrations of 1 or even 10 μg per cubic centimeter; and there is a final group which is not killed by penicillin in any reasonable concentration Moreover, these differences are not predictable, since different strains of the same species, not previously exposed to penicillin, may vary widely in their susceptibility. One may reasonably assume that the less susceptible a given organism is to penicillin *in vitro*, the higher will be the blood and tissue level which must be attained in order to kill those organisms *in vivo* The maintenance of the higher blood levels requires more frequent injections of penicillin, or its administration in larger dosage; and in general, the dosage schedule of penicillin must be adjusted to the susceptibility of the particular organism concerned.

PENICILLIN ACTIVITY AGAINST A GIVEN STRAIN

The terms "susceptibility to penicillin" and "inhibiting concentration of penicillin" require somewhat more precise definition With every organism so far studied in this respect, one may define three different "effective" concentrations of penicillin (18–20).

(*1*) The first is that concentration which significantly reduces,

but does not prevent, the multiplication of the organisms. This is illustrated by curve 0 004 of Figure 3, which describes the direct bactericidal action of penicillin G on the C-203 strain of hemolytic streptococcus.

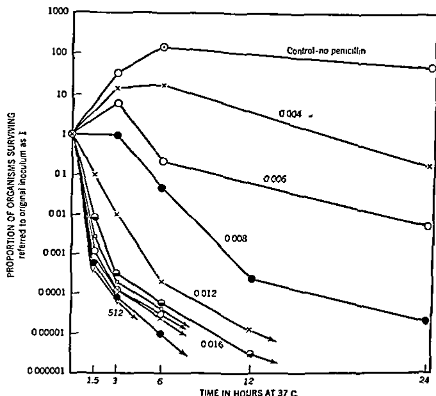


Fig 3 Effect of penicillin G concentration on rate of its bactericidal action against Streptococcus pyogenes, strain C-203 (18). Figures on curves are in micrograms per cubic centimeter.

(2) At a somewhat higher concentration of penicillin (e.g, concentrations of 0.006 or 0 008 μg per cubic centimeter in Figure 3), *there is a progressive decline in the number of variable organisms.* This concentration, which effects a net bactericidal action, approximates the bacterial "susceptibility" as ordinarily determined.

(3) Beyond that minimally effective level, however, *the rate of bactericidal action increases strikingly with the concentration of*

penicillin. Thus, in the experiment illustrated in Figure 3, there were more than 1,500 times as many organisms surviving after 6 hours exposure to 0 008 μg. per cubic centimeter than there were after similar exposure to 0 048 μg; and to kill 99 9 per cent of the bacteria required less than $1^1/_2$ hours at the higher concentrations, as compared with $9^1/_2$ hours at the lower. However, one soon attains a *maximally effective level*, which with this strain of streptococcus

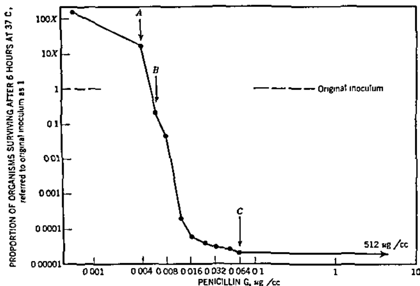


Fig 4 Effect of penicillin G concentration on proportion of Streptococcus pyogenes (C-203) surviving after 6 hours at 37 C Inoculum = 4.3×10^5 per cubic centimeter A Concentration which reduces rate of multiplication B: Concentration with barely detectable but significant bactericidal action C: Concentration with maximal bactericidal effect, not further increased at higher concentrations of penicillin (18)

proved to be 0 064 μg per cubic centimeter Even an increase of 30,000 times in the concentration of penicillin beyond this maximally effective level, to as high as 2,048 μg per cubic centimeter did not significantly accelerate the rate at which the organisms were killed

These relationships are more clearly illustrated in Figure 4, which again relates to the streptococcidal action of penicillin G Points A, B, and C in the figure are the three "effective" levels of concentration as defined in the preceding paragraph. that which suffices merely to

TABLE II
"Effective Levels" of Penicillin G for a Number of Bacteria (18)

Infecting organism	Penicillin G concn. (μg./cc.)* to: Reduce growth rate	Kill organisms slowly	Kill organisms at max rate	Hours to kill 99 9% of organisms at optimal penicillin concns	Per cent of organisms surviving after 6 hrs exposure to maximally effective penicillin concns.
Streptococcus pyogenes (C-203)	0 004	0 006–0 008	0 064	1 5-2	0 002–0 004
Pneumococci (types 1,3,8,12,14, 24)	0 008–0 012	0 024	0 064	3–5	0 03 ±
Staphylococcus aureus (6 susceptible strains)	0 016–0 024	0 024–0 064	0 064–0 25	5–20	0 05–1 0
(1 resistant strain)	0 25	1	16	11	6
Treponema pallidum (Reiter)	0 016	0 032	1 ±	25–35	5–10
Streptococcus faecalis (5 susceptible strains)	1	2–4	4–6	5	0 05
(2 resistant strains)†	1	3–4	4–6	>48	10–50

* To transform to units, multiply by factor 1 7 (1 mg. = 1667 units).
† Resistant in terms of death even at optimal concentrations of penicillin.

reduce the net rate of multiplication, that which effects a progressive decrease in the number of viable organisms, and that which kills the organisms at a maximal rate, not affected by a further increase in concentration.

As is summarized in Table II and Figure 5, qualitatively similar results have been obtained with every organism so far studied in this respect. Whether with pneumococci, Streptococcus faecalis,

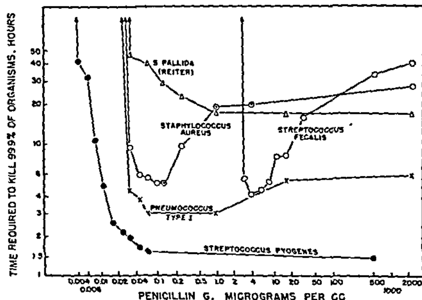


Fig 5 Time required for penicillin G at various concentrations to kill 99.9 per cent of several bacterial species. Each curve summarizes a single experiment with a single strain (18)

Streptococcus pyogenes, Staphylococcus aureus and albus, or Treponema pallidum, one can define minimally and maximally effective bactericidal concentrations of penicillin These varied to an extraordinary degree even among these five species, as did also the maximal rate at which the organisms could be killed by penicillin under the most favorable conditions. Thus, the maximally effective concentration varied from 0.064 µg. (0.1 unit) per cubic centimeter in the case of the C-203 strain of Strep. pyogenes to 4 to 6 µg. (7 to 14 units) per cubic centimeter in the case of Strep. faecalis; the pro-

portion of organisms surviving after 6 hours' exposure at the optimal concentration varied from 5 to 10 per cent in the case of spirochetes to as little as 0 002 to 0 004 per cent in the case of Strep. pyogenes; and the minimum time within which penicillin could kill 99.9 per cent of the organisms varied similarly from less than 2 hours with Strep. pyogenes to 25 to 35 hours with T. pallidum. It is particularly to be noted that the maximal rate at which an organism could be killed by penicillin bore no necessary relationship to the concentration necessary to produce that maximal effect.

However else penicillin effects cure, its direct bactericidal action is of obvious importance. It follows that the penicillin concentration at the focus of infection should be kept at definitely bactericidal levels, and preferably at the maximally effective level, whatever that concentration happens to be for the particular organism concerned (column 4 of Table II) If this optimal concentration of penicillin could be maintained at the focus of infection, the time necessary to effect a cure might then be determined in part by the maximal rate at which the particular organism can be killed by penicillin.

PARADOXIC ZONE REACTION WITH EXCESS PENICILLIN

Tissue concentrations of penicillin greatly in excess of the maximally effective level as defined in the preceding section may represent waste penicillin. If the organism is most effectively killed by 0 1 μg. per cubic centimeter, it probably does not accelerate the cure of the infection to maintain concentrations 10 or 20 times higher. The advantage of very large doses of penicillin consists not in the higher concentrations thereby attained in the body fluids but in the longer time for which it then remains at effectively bactericidal levels (see page 120). A complicating factor, however, is the fact that, as shown in Figure 5, with at least some organisms (some strains of Staph aureus and Strep. faecalis), high concentrations of penicillin may actually be less effective than lower concentrations (21). Thus, with one strain of Staph. aureus, there were 50 to 200 times as many surviving organisms after 6 hours exposure to penicillin G at 10 to 2,048 μg. per cubic centimeter than there were after similar exposure to 0 064 μg. per cubic centimeter. It required 20 to 25 hours to kill 99 9 per cent of the organisms at the high concentrations, as against approximately 5 hours at the lower concentration

This paradoxic zone reaction, as yet unexplained, has been observed with 5 of 7 strains of Strep faecalis and with 4 of 9 strains of Staph. aureus and albus (21). In infections caused by these strains, tissue concentrations of penicillin higher than the optimal levels indicated in Table III might well be less effective thera-

TABLE III
Renal Clearance of Penicillins F, G, K, and X in Man and in Rabbits (54)

Penicillin species	Cubic centimeters of blood cleared of penicillin by kidney, per minute					
	Man				Rabbit	
	Observed clearances after					
	Continuous intravenous infusion	Single intramuscular injection, aqueous	Single injection, oil–beeswax	Mean clearances, cc	Observed clearances after single intramuscular injection, aqueous	Mean clearances, cc
F	550,900	—	—	725	31,37,15,46	32
G	525	335,850	687,409	560	111,24,54, 70,55,17, 98,44,24, 65	56
K	272,269	225,188, 627,375, 165	—	300	—	—
X	652	920,800, 542,865, 450,763	—	710	25,40,36, 54,23	36
Commercial penicillin	—	—	—	—	53,57,40, 28,42,24, 43	4 1

peutically, and overdosage might actually delay cure. To date, this zone effect has been observed only with these two species. It was not observed with the C-203 strain of Strep pyogenes, 7 stains of pneumococci of as many different types, or with the Reiter strain of cultured treponemata With these organisms, high concentrations had the same bactericidal activity as the relatively low optimal values indicated in Table III

PENICILLIN RESISTANCE

It is necessary to emphasize that almost all the work on the resistance of bacteria to penicillin has dealt with only one aspect of that resistance, measured by the concentration of penicillin necessary to kill a given bacterial suspension There is, however, another

measure of resistance, also of therapeutic importance, and manifested at the rate at which a given strain can be killed by penicillin, even at optimal concentrations. This rate of bactericidal action varies between wide limits, and helps determine the total time for which treatment must be continued in order to effect cure.

Four distinct types of bacterial resistance have now been described

Species and Strain Differences Some bacterial species are sensitive to as little as 0 004 μg. per cubic centimeter, while others will grow luxuriantly at 1,000 times that concentration and die only when exposed to 10 or 20 μg. per cubic centimeter. With some organisms (22–24) but not all (18,25–27), the resistance to penicillin and the necessity for high concentrations is associated with the production of an extracellular enzyme, penicillinase, which inactivates the drug.

As previously indicated, bacterial species, and different strains of the same species, may differ not only in the concentration of penicillin necessary to kill, but also in the rate at which they are killed at that concentration Sterilization of a culture, or the cure of an infection, may take hours with one organism and days in another

Population Variation. In the same species, and even in the same culture, it is obvious that all the organisms are not equally susceptible to penicillin (*1*) Individual bacteria vary widely with respect to the minimal lethal concentration of penicillin It is partly for this reason that the rate at which a bacterial suspension is killed by penicillin increases so strikingly with the concentration of the drug within a certain critical range It is for this reason also that a culture exposed to threshold concentrations of penicillin may grow out luxuriantly after an initial period during which most of the organisms may have been killed The surviving, relatively resistant organisms then multiply faster than they can be killed at that concentration, so that the net number of viable organisms increases almost exponentially It is therefore necessary to have, at the focus of infection, not merely the concentration of penicillin which kills some of the organisms, but a concentration sufficiently high eventually to kill all the organisms, susceptible and resistant alike (*2*) Individual bacteria differ markedly in the rate at which they are killed, even at maximally effective concentrations of penicillin Thus, in a suspension containing 1,000,000 organisms per cubic centimeter 99 per

cent may be killed in 1 hour; but it may take 12 to 24 hours to kill the very last organism.

These wide differences in the susceptibility of individual bacteria in a given culture are not necessarily genetic in character. With most of the organisms so far studied, subcultures of the last few surviving cells are killed by similar concentrations of penicillin at the same rate as the original culture.

Growth Phase Resistance. A third type of resistance to penicillin, and one which has not been satisfactorily explained, is the fact that the organisms are vulnerable to penicillin only during the period of active growth (23,28,29). This probably explains the failure of penicillin to kill bacteria at low temperatures (28,30,31), it may explain its enhanced activity at temperatures above 37 C., both *in vitro* (30,32,33) and *in vivo* (34), and may explain also the fact that heavy suspensions of bacteria, in which the number of viable organisms has stabilized or is decreasing, are also unaffected by the drug (31). This puzzling phenomenon may provide a clue to the mode of action of the drug.

Bigger (29) has suggested that some treatment failures may be due to survival of small numbers of organisms which happen to be in a nondividing phase; and that intermittent treatment, in which penicillin is deliberately withheld in order to permit the multiplication of such "persisters," may be more effective than continuous treatment This important and provocative suggestion deserves careful consideration, particularly in infections such as early syphilis, in which failure rates of 5 to 15 per cent are reported (35,36).

Penicillin Resistance "Acquired" after Exposure to the Drug. The aspect of penicillin resistance which has been most intensively studied, and the one of greatest therapeutic significance, is the possibility that when bacteria are exposed to penicillin, those which survive may have become resistant to the drug. In patients, and depending on the rate at which this resistance develops, this would mean that larger and larger doses would be necessary to continue the bactericidal action to the point of cure; and in the course of repeated patient-to-patient transfers, the organism may become prohibitively resistant Whether such drug resistance is an adaptive but hereditable change in the bacteria "induced" by exposure to the drug, or whether it occurs as a spontaneous mutation, the drug acting merely as a selective factor which kills off the susceptible

organisms and thus permits the propagation of the resistant mutant, is a moot problem which is not within the scope of the present paper. The great bulk of evidence favors the latter view (20,37).

To date, such "acquired" penicillin resistance has been considered only in relation to that concentration of penicillin necessary to kill the organism. The possibility that there may be a second type of resistance, measured by the rate at which the organisms can be killed, has not been studied.

Fortunately, penicillin-resistant mutants are apparently thrown off at a low rate, and the degree to which resistance is stepped up with each single mutation is usually small. Thus, with Strep. pyogenes, pneumococci, Strep. faecalis, or T. pallidum, if one begins with 1,000,000 organisms and incubates with penicillin until all but 1 to 10 have been killed, the daughter cells of these few remaining bacteria are not demonstrably more resistant to penicillin than the original culture (38). Although those few remaining organisms clearly are by far the most resistant in the original suspension, that resistance apparently reflects only the normal variation in susceptibility in a bacterial population, and is not necessarily indicative of a genetic or adaptive change In consequence, it is necessary to carry out repeated transfers in mediums containing gradually increasing concentrations of penicillin, in order to arrive eventually, after a series of stepwise mutations, at a strain which is many times more resistant than the original organism. Thus Bahn, Ackerman and Carpenter (39), by transferring gonococci every 48 hours into mediums containing progressively higher concentrations of penicillin, had after 32 weeks increased the resistance of 5 different strains by 8, 64, 128, 200 and 400 times. The resistance was measured in terms of the maximal concentration of penicillin which permitted growth There were no data as to whether the organisms simultaneously developed resistance in terms of the rate at which they could be killed by the drug in maximally effective concentrations In the course of 98 transfers, Miller and Bohnhoff (40) increased the penicillin resistance of gonococci from 0 06 unit per cubic centimeter to 7 to 21 units per cubic centimeter, a one hundred to three hundred fold increase McKee and Houck (41) by 58 to 60 similar passages effected a thirty fold increase in the resistance of Strep. pyogenes (C-203), a sixfold increase in type I and type II pneumococci, but a six thousand fold increase in that of Staph. aureus Meningococci (42,43), α and β hemolytic streptococci (42), and a number of other

strains have similarly been rendered resistant by a large number of transfers in progressively increasing concentrations of penicillin

Staphylococci differ sharply from most other organisms so far studied in the ease with which they can be rendered resistant to penicillin, that is, in the frequency with which resistant mutants appear, and in the magnitude of the difference in resistance for each successive mutation In 60 such transfers, McKee and Houck (41) were able to increase the resistance of their organisms six thousand fold. A number of other changed properties have been found to be associated with the increased resistance, thus indicating that the resistance to penicillin is only one manifestation of a mutation which may affect numerous vital functions of the cell Morphologic (40,44) and metabolic changes (39), and decreased virulence (40, 43) have all been reported, although of course not all for the same species. The ability of the resistant mutant to produce extracellular penicillinase is a frequent, but not a necessary, concomitant of the resistance (45). In such instances, it is probably causally related to the observed resistance; but for many strains some other mechanism is responsible. The resistance to penicillin is usually maintained on further cultivation in penicillin-free mediums, but in some species, it goes down progressively (40,46,47), perhaps due to reverse mutations (37). It is pertinent to note that mutant strains resistant to penicillin G are reported to be equally resistant to penicillin X (42)

Corresponding to the ease with which resistant strains of staphylococci can be isolated *in vitro*, this organism has repeatedly been observed to become resistant in the course of treatment (42,46–48) Unlike the resistant strains produced *in vitro*, the cell bodies of the strains which acquire resistance *in vivo* have been found to contain a potent inhibitor of penicillin (46,49) They further differ from the *in vitro* strains in the greater stability of the penicillin resistance on subsequent repeated subculture in penicillin-free mediums (46–48)

Penicillin-resistant strains have been observed to develop during the treatment of infections other than staphylococcic (42) However, the evidence to date indicates that this will not be the rapidly developing and widespread phenomenon which eventually so largely vitiated the therapeutic utility of the sulfonamides in the treatment of gonorrhea for example, and which promises to modify seriously

the usefulness of streptomycin. The physician can help retard the appearance and propagation of these penicillin-fast mutants (*1*) by effective and adequate treatment, continued until the last organism has been killed and (*2*) by using larger doses of penicillin, and, in particular, (*3*) by using other chemotherapeutic agents to supplement penicillin, when that drug fails to effect a cure in a normally susceptible infection.

Pharmacologic Properties of Penicillin Modifying Concentrations in Tissue Fluids

RATE OF URINARY EXCRETION AND RENAL CLEARANCE

When penicillin G is injected intramuscularly in aqueous solution, most of the drug is quickly absorbed, and within less than 15 minutes the serum concentration reaches a peak value which is roughly proportional to the amount of penicillin injected Thereafter, the drug rapidly disappears from the circulating blood at the rate of opproximately 70 per cent an hour, as illustrated in Figure 6 (50). The somewhat slower rate of fall at higher dosages, evident in the top curves of Figure 6, are discussed in a later section. In consequence of this rapid disappearance of penicillin from the blood and tissues, injections must be repeated at frequent intervals, determined by the size of the individual injection and the serum level which one is attempting to maintain.

Some of the factors which affect the rate at which penicillin disappears from the blood, and the absolute concentrations attained in the blood and tissues, are discussed in the following sections.

Penicillin X disappears from the blood at a significantly slower rate than does penicillin G or F (5,14), while penicillin K in low dosage disappears much faster (7,8,14) The pharmacologic basis for these differences, and their therapeutic significance are discussed in a following section.

RATE OF URINARY EXCRETION AND RENAL CLEARANCE OF PENICILLINS F, G, K, AND X

The factor which is primarily responsible for the rapid disappearance of penicillin from the blood and tissues after its intramuscular injection in aqueous solution, and which most seriously limits its

therapeutic activity, is its rapid excretion in the urine (51,52). In man, from 59 to 88 per cent of penicillins F, G, and X appeared in the urine within 1 hour after their intramuscular injection, and a total of 65 to 97 per cent was excreted in 4 hours (7,8,14). With penicillin K, however, the urinary recovery in man after its injection at 0 6 mg. per kilogram of body weight varied between 15 and 39

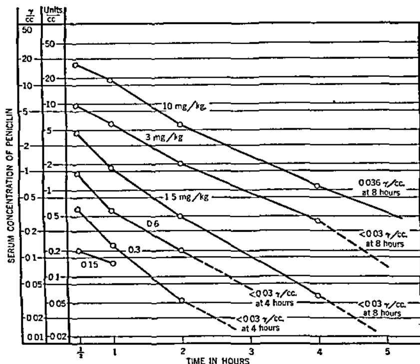


Fig 6 Serum levels of penicillin G in man after intramuscular injection of aqueous solution Each point in the figure is the median of at least 10 determinations All results have been corrected for the error introduced by the serum binding of penicillin (50,50a)

per cent, averaging 28 per cent (14). The lower recovery from the urine of penicillin K is probably due to its inactivation *in vivo,* as discussed in the next section.

The rapid excretion suggested an active tubular secretion A number of workers (51–53) have found the renal clearance of crude penicillin to approximate that of sodium hippurate, and to be several

times the glomerular filtration rate. This has also been found true of the crystalline penicillins F, G, and X (Table III, p. 115). Both by glomerular filtration and tubular secretion the kidney abstracts and excretes essentially all the penicillin which reaches it via the blood Indeed, the renal clearance of penicillin, calculated from simultaneous determinations of the plasma concentration and the rate of urinary excretion, can be used as a measure of renal plasma flow and renal function The rapid fall in the blood level, which averaged 70 per cent (of the residual penicillin) per hour and 2 per cent per minute with penicillin G injected in dosages of 0 6 mg. per kilogram of body weight (14,50,50a), largely reflects its almost total clearance by the kidney. The paradoxically low renal clearance of penicillin K (54) is due at least in part to its binding by serum protein as described by Tompsett, Shultz, and McDermott (17).

Several methods have been suggested to delay the excretion of penicillin, none wholly successful. The limitation of water or salt intake should have no significant effect on the excretion of a compound which is completely cleared by the kidney at a rate independent, within wide limits, of the blood level or rate of urinary flow (54). The retardation of excretion by saturating the renal secretory mechanism with compounds such as sodium aminohippurate (56, 57) is physiologically sound, since apparently the same mechanism operates with these compounds as is responsible for the rapid secretion of penicillin. The method has the disadvantage of requiring the continued administration of the blocking agents, for they are excreted as fast as penicillin itself; under such circumstances, one might just as well repeat the injection of penicillin instead This objection may not apply to the recently discovered agent "caronamide" (58), which apparently retards the excretion of penicillin by a specific action on the secretory mechanism. However, with this agent, also, repeated doses by mouth are necessary in order to prolong the inhibitory effect on penicillin excretion

If it were possible so to modify penicillin chemically as to reduce its tubular secretion and renal clearance, the blood and tissue levels would then be maintained for longer periods of time, and a given dosage would be correspondingly more effective therapeutically. To date, however, the only penicillin shown to have a renal clearance significantly lower than the total renal plasma flow is penicillin K In the case of this penicillin, however, the low clearance is probably

related to its binding by the serum proteins, and is associated with an increased susceptibility to inactivation, both of which seriously impair its therapeutic activity.

THE ABSORPTION OF PENICILLIN

Penicillin in Oil and Beeswax. As has been discussed, the rapid urinary excretion of penicillin, and its consequent rapid disappearance from the blood, effectively limit the time for which it remains at bactericidal levels in the blood and tissues. One method of pro-

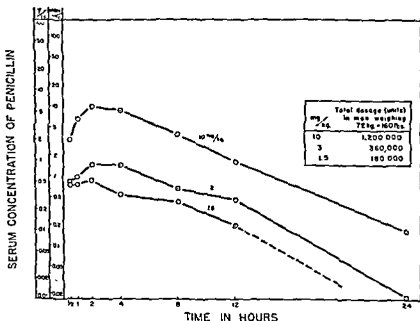


Fig 7. Serum levels of penicillin G in man after injection of 3 readily absorbed peanut oil–beeswax suspensions (Lots A–C) Each point in the figure is the median of at least 10 determinations All results have been corrected for the error introduced by the serum binding of penicillin (50).

longing its time of action would be to delay its absorption. A relatively insoluble modification of penicillin which is slowly absorbed from an intramuscular depot might provide effective levels for longer periods than is the case with highly soluble penicillins now in use. A penicillin modification sufficiently insoluble for this purpose has

not yet been prepared. Romansky and Rittman (59) have, however, demonstrated that penicillin suspended in peanut oil and beeswax is absorbed, after intramuscular injection, more slowly than it is from an aqueous solution. The blood concentration therefore does not reach as high a peak value, but is maintained at effective levels for much longer periods The Ca salt of amorphous penicillin was originally used for this purpose, but both the sodium and potassium salts of crystalline penicillin G have since been sucessfully incorporated in such suspensions.

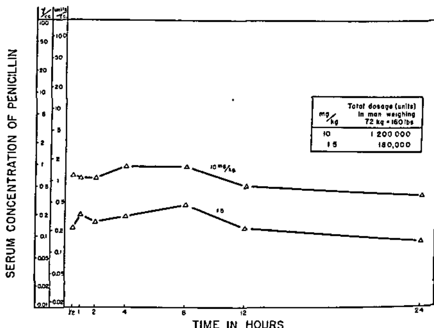


Fig 8 Serum levels of penicillin G in man after injection of a slowly absorbed peanut oil–beeswax suspension (Lot D) Each point in the figure is the median of 6 to 8 determinations All results have been corrected for the error introduced by the serum binding of penicillin (50).

The degree to which the blood levels may be prolonged is indicated in Figures 7 and 8, as compared with the curves in Figure 6. There are several aspects of these curves which are worthy of comment at this point:

(*1*) The peak concentration afforded by penicillin in oil and

beeswax is much lower than that observed when the same dosage is injected in aqueous solution (Fig. 9).

(2) Although the average curves fall off slowly and regularly, the absolute serum concentrations in individual patients may show wide variations (50).

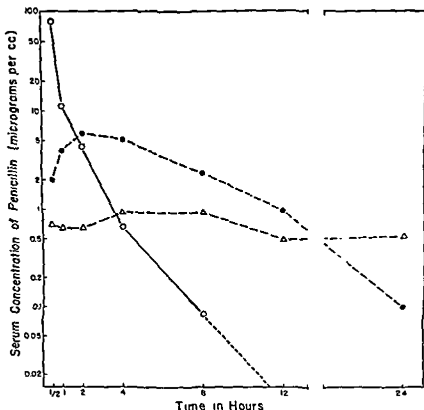


Fig 9 Contrasting serum levels of penicillin afforded by aqueous solutions (O), readily absorbed peanut oil-beeswax suspensions (Lots A-C) (●), and slowly absorbed peanut oil-beeswax suspension (Lot D) (△).

(3) Different lots of penicillin suspended in peanut oil and beeswax, and containing the same amounts of penicillin, do not necessarily behave similarly on injection The particle size of the penicillin, the particle size of the beeswax, the fluidity of the suspension, and perhaps other factors as well, modify the rate of its absorption

and the resulting serum penicillin levels to an important degree. This is clearly shown by the differences between Figures 7 and 8 Figure 7 summarizes the results with three lots of penicillin, in all of which the material was said to be finely micronized. For the patients of Figure 8, however, a new preparation was used in which the penicillin particles were said to be significantly larger. It is apparent that the three preparations represented in Figure 7 were quickly absorbed, the blood levels reaching a maximum in 2 hours, and thereafter falling off rapidly. None of the patients receiving 1 5 mg. per kilogram (an average total of 180,000 units) had measurable blood levels 24 hours after the injection, and at dosages of 10 mg per kilogram (an average total of 1,200,000 units), the median serum concentration after 24 hours was 0.1 μg per cubic centimeter. In marked contrast, the preparation used for the experiments of Figure 8 was so slowly absorbed as not to give a sharp peak, the maximum blood levels were not reached for approximately 8 hours, and the serum concentration fell off only slowly thereafter Every patient receiving 1 5 mg per kilogram had significant amounts of penicillin in the serum 24 hours after the injection, and the median serum level at that time (0 14 μg /cc) was greater than that provided by 10 mg. per kilogram of the other penicillins.

(*4*) Romansky (60) has recently shown that the time for which peanut oil–beeswax suspensions of penicillin provide demonstrable blood levels is significantly prolonged if the injections are given in the late afternoon or evening rather than in the morning This presumably reflects the fact that after an evening injection there is relatively little motion massage of the injected area during the following 12 hours, and correspondingly slower adsorption. A larger proportion then remains at the site of injection for absorption during the following 12 hour period However, this difference between morning and evening injections is significant only with preparations, which, like those used for patients of Figure 7, are absorbed fairly rapidly. With preparations which are absorbed only slowly (Fig. 8), one does not find a significant difference between morning and evening injections with respect to the magnitude of the 24 hour levels (50).

(*5*) One final point should perhaps be stressed The incorporation of penicillin in oil and beeswax enhances its therapeutic activity only by virtue of the fact that it prolongs the time during which the

drug remains in the blood and tissues at effectively bactericidal levels. A similar effect would be produced by giving larger doses in aqueous solution, or by repeating the injection of the aqueous solution at shorter intervals. Thus, with the dosage of penicillin in oil and beeswax most often used (300,000 units = 180 mg. = an average 2.5 mg./Kg.), a serum level of 0.5 unit is provided for a period of approximately 5 to 10 hours, depending on the type of preparation used. To provide that same level for 6 hours with an aqueous solution for example, would require either a single injection of approximately 1,600,000 units, 2 injections of 300,000 units each at 3 hour intervals, *4 injections of 150,000 each at 1.5 hour intervals, or 6 injections of* 80,000 units each at 1 hour intervals (50). To produce equally lasting blood levels with a single injection one would have to give at least 5 and perhaps 10 times as much penicillin in aqueous solution as is necessary in peanut oil–beeswax suspension but this disproportion between the aqueous solution and oil suspension is progressively reduced as the aqueous material is progressively subdivided into a larger number of injections.

The same relationships have been shown to apply in the thera-[illegible] of penicillin. Thus, in the treatment of experimental [illegible] rabbits may be cured by a single [illegible] per kilogram of body weight. [illegible] ith a single injection of penicillin in aqueous solution [illegible] an 600,000 units per kilogram, or more than 12 times as much. This curative dose of aqueous penicillin was, however, reduced to a total of 50,000 units per kilogram if the penicillin was divided into 4 daily injections, to 1,770 units per kilogram with 16 injections given twice daily, and to a total of only 360 units per kilogram if the penicillin was subdivided into 50 injections at 4 hour intervals. On the latter schedule, penicillin in aqueous solution was 150 times as effective, unit for unit, as a single injection of penicillin in oil and beeswax.

In deciding on a schedule of treatment, the physician may therefore choose between (*1*) a relatively small number of injections of the peanut oil–beeswax suspension, (*2*) a similarly small number of injections of penicillin in aqueous solution, but at much larger dosage, or (*3*) a large number of injections of aqueous penicillin, each in relatively small dosage, and to a smaller aggregate total. The choice involves the following factors: (*1*) the cost of the penicillin; (*2*)

the cost of the hospitalization necessary for a schedule of repeated injections of aqueous solution, but not necessary with the oil-beeswax suspension, or with large doses of penicillin in aqueous solution given once or twice daily; and (3) the relative discomfort of the three schedules to the patient and their relative inconvenience to the physician.*

Absorption after Administration by Mouth. It has been shown that penicillin is absorbed after its administration by mouth, and that such absorption is facilitated by the simultaneous administration (in the same tablet) of an alkalinizing agent such as sodium citrate or calcium carbonate to counteract the destructive effects of the gastric acid on the penicillin (63).

An average of 20–25 per cent of the penicillin is absorbed, and one must therefore give 4 to 5 times as much penicillin by mouth as by injection to achieve blood levels of the same magnitude or duration (64–68). These are, however, average figures The degree of absorption is highly irregular, and in some patients negligible. It follows that with patients who are so sick that the provision of an effective level for long periods of time is of critical importance, reliance cannot be placed on the peroral administration of the drug.

Absorption after Inhalation Taplin (69) has recently reported that finely micronized penicillin administered by inhalation results in a prolonged absorption and sustained blood levels, comparable to those obtained on the intramuscular injection of aqueous solutions.

THE DISTRIBUTION OF PENICILLIN IN THE BODY

In the therapeutic administration of penicillin, due provision must be made for the fact that penicillin is not uniformly distributed in the body Penicillin reaches the tissue fluids by diffusion out of the

* Since this manuscript was written, a new preparation of penicillin has been devised which has gained wide clinical acceptance and which is apparently superior to POB as an absorption-delaying vehicle This new preparation consists of the relatively water-insoluble procaine salt of penicillin G suspended in oil, with the addition of aluminum monostearate, which further delays the absorption of the penicillin With this preparation, the majority of the patients injected have measurable serum concentrations of penicillin, i e, in excess of 0 03 microgram per cubic centimeter, for more than 72 hours after a single intramuscular injection of 1 cc containing 300,000 units, i e, 180 mg of penicillin G

blood. This continues only so long as the concentration of freely diffusible penicillin in the plasma exceeds that in the tissue fluids; thereafter the process is probably reversed, the penicillin then leaving the tissues to return to the blood.

Because of the speed with which penicillin is absorbed after its intramuscular injection in aqueous solution and because of its rapid urinary excretion, the blood level curve is characterized by a sharp rise and a rapid fall (Fig. 6). In consequence, at foci which are poorly supplied by blood, or only slowly equilibrated with it (e g., organized thrombi on a heart valve in cases of subacute bacterial endocarditis; empyema fluid) the maximum penicillin concentration afforded by a single injection may be only a fraction of that attained in the circulating blood; and concentrations effectively bactericidal for the particular organism may be provided for a relatively short period of time.

In order to provide effective concentrations at such foci, it becomes necessary either to give large injections of penicillin, or, by giving repeated injections at sufficiently frequent intervals, to *maintain* the plasma concentration over a long period of time and thus permit the continuing slow diffusion of penicillin into the focus of infection Even under such circumstances, no matter how long this process of equilibration were permitted to continue, the concentration of penicillin in the tissue fluids might never attain that in the plasma. The first limiting factor is the combination of penicillin with the plasma proteins, as discussed in the following section. In the case of penicillin G, approximately half the penicillin (55,17) is thus bound, leaving only half of the drug as a freely diffusible fraction available for distribution at any one moment. In the second place, the inactivation of penicillin by the tissues themselves, or the reversible combination of penicillin with tissue components, may further limit the concentration of free and reactive penicillin in the tissue fluids (15, 16,70).

Surprisingly little specific information is available as to the actual concentration of penicillin in various tissues and tissue fluids, compared to that in the plasma Further, such data as are available must be interpreted in the light of the fact that the diffusion of penicillin into and out of the tissues is a dynamic process: the relative concentrations in the plasma and tissues at any one moment

must be considered in relation to the method of administration and the time of the observation

Only minimal amounts of penicillin reach the cerebrospinal fluid (71–73) or saliva (71), but the concentrations in ascitic or pleural fluid, in subcutaneous edema fluid (73), and in the joint in cases of hydrarthrosis (74) have been found to be comparable to those in the serum. The drug has been shown to pass through the placenta, the concentrations in the cord blood generally being lower than those in the maternal circulation (75) Wide variations have been reported in the penicillin concentration of individual tissues (15,76,82). In dogs, the plasma:tissue ratios for the brain, muscle, spleen, lungs, liver, and kidney have been found to be 47, 6, 6, 2, 8, and 0 55, respectively (15). Whether the penicillin which can be demonstrated in organ emulsions consists solely of drug present in the tissue fluids, or whether some of the reactivity represents drug which had either diffused into the tissue cells, or which had been bound by the cells without necessarily having been inactivated by that combination, remains to be determined.

SERUM BINDING OF PENICILLIN

Penicillin has been shown (55,17) to be bound by the serum proteins In the case of penicillin G, approximately 50 per cent of the penicillin is thus bound, so that the concentration available for diffusion at any one amount is approximately half the total plasma concentration. In the case of penicillin K, more than 90 per cent of the penicillin is bound by the serum proteins, and the freely diffusible fraction averages only 10 per cent of the total.

It must be emphasized that this binding of penicillin by the serum proteins is not synonymous with its inactivation, discussed in the following section The binding reaction takes place even at icebox temperatures (38), at which no inactivation of penicillin, whether of F, G, K, or X, can be demonstrated (13), and is reversible When serum is diluted, there is approximately a linear decrease in the

t

that the renal clearance of penicillins F, G, and X approximates the total renal plasma flow indicates that the tubular secretory mechanism can remove from the plasma both the free and bound fractions of these three penicillins On the other hand, the low value for the

renal clearance of penicillin K, which averages one-half that of the other penicillins, suggests that the tubular secretory mechanism may not be able to dissociate this penicillin from its combination with plasma protein, either because a larger proportion is bound, or because it is bound more firmly than are the other three penicillins.

The serum binding of penicillin is of therapeutic importance, inasmuch as the concentration of freely diffusible penicillin available for distribution at any one moment is decreased to the degree that it has been bound. With penicillin G, at least a twofold excess must be provided in the plasma to compensate for this binding, in order to deliver the desired concentration to the focus of infection. In the case of penicillin K, the almost complete binding by serum protein has two opposing effects. The fact that 90 per cent is bound limits by that much the proportion instantaneously available for diffusion into the tissues, and to that extent curtails therapeutic activity. On the other hand, the lowered renal clearance, probably caused by this binding, permits the retention of penicillin in the body for longer periods, and under appropriate experimental conditions might make for actually enhanced therapeutic activity.

The binding of penicillin by serum protein also affects the biologic assay of the penicillin content of serum (13,14,17,77,78). The apparent penicillin content of a given serum, judged by its bactericidal action *in vitro*, will be less than its actual penicillin content to the degree that the drug had been bound by the serum protein and was therefore not free to act on the organisms The similar but quantitatively less important error introduced by the inactivation of penicillin under the conditions of the test is discussed below. Because the proportion of penicillin bound or inactivated varies with the concentration of serum, the magnitude of the serum error in biologic assays which involve the use of serial serum dilutions will depend on the concentration of serum in the indicator tube of the assay. Thus, Figure 10 shows the degree to which the serum concentration was found to affect the results in the technic of penicillin assay used in this laboratory It required an average of three times as much penicillin G to inhibit hemolysis in the presence of whole human serum as in its absence (65 per cent inhibition by serum), in the case of penicillin K, it required 18 times as much (94 5 per cent inhibition) With penicillin G, the serum error was negligible at serum concentrations of 1·8 or less; with penicillin K, it became negligible only at

concentrations of 1·32 or less Figure 11 gives average corrective factors by which the apparent concentration of penicillin must be multiplied in order to compensate for the inhibitory effect of serum in the assay. It must be emphasized that Figure 10, and the corrective factors in Figure 11, are applicable *only to the particular technic*

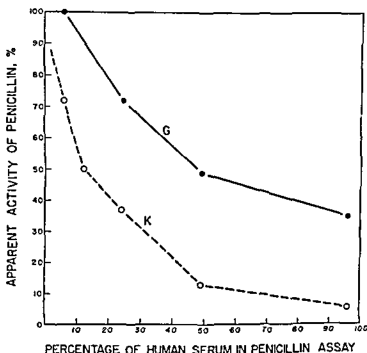


Fig 10 Inhibitory effect of human serum in the bioassays of penicillins G and K (78) Serial dilution technic, with Strep pyogenes *(C-203) as test organism (13,54).*

used in those experiments Similar charts, however, can be developed for any method of biologic assay in which serial dilutions of serum are used.

INACTIVATION OF PENICILLIN BY PLASMA AND TISSUES

Superimposed on the rapid urinary excretion of penicillin, and contributing to its disappearance from the blood and tissues, is the

fact that it is inactivated by serum or plasma (13), and perhaps by the tissues as well (15,16,70).

Serum Inactivation. The inactivation of penicillins F, G, and X by plasma or serum *in vitro* is a slow reaction caused by a serum constituent stable at 56 C., and which, independent of the concentration of penicillin, has been found to destroy 7 to 13 per cent per

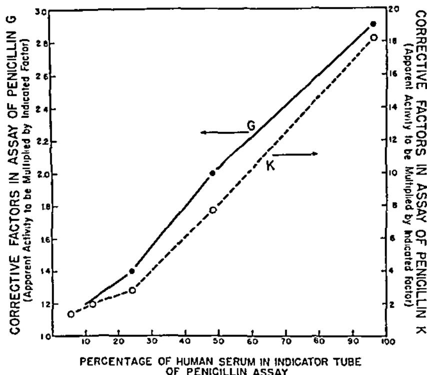


Fig 11 Corrective factors for the inhibitory effect of human serum in the bioassay of penicillins G and K (78).

hour (13). Penicillin X is inactivated somewhat more slowly than either F or G This, perhaps coupled with a similar resistance to inactivation by tissue, may contribute to the slightly but significantly higher and more prolonged blood levels afforded by this species of penicillin in rabbits and in man (5,14).

Penicillin K, on the other hand, is inactivated by serum or plasma much more rapidly than F, G, or X (13). Two apparently distinct

mechanisms are concerned. One involves the same thermostable component which slowly inactivates F, G, and X. In addition, there is a thermolabile serum component which inactivates only penicillin K, at a rate which varies inversely with the concentration of the drug In consequence, when penicillin K is added to serum in low concentrations, on the order of $^1/_2$ to 2 μg. per cubic centimeter, its activity falls off more rapidly than that of F, G, or X. In high concentrations, however, penicillin K is inactivated at a rate which does not significantly differ from that of the other penicillins The inactivating mechanism which is specific for K is apparently saturated by relatively small concentrations of penicillin

The possible, and indeed probable, relationship between the rapid and reversible binding of penicillin by plasma protein, and the slow irreversible inactivation of penicillin by plasma, remains to be determined.

The inactivation by serum of penicillins F, G, X, and in particular of K, may have a significant effect in biologic assays of the penicillin content of serum (see page 131) Small concentrations of penicillin K may be completely inactivated in a few hours, and before all the organisms used as inoculum in the biologic assay had been killed. The actual penicillin content of the serum would therefore be significantly greater than the *in vitro* test would indicate. This error is, however, significant only on specimens which contain small amounts of penicillin, requiring the use of whole, 1 2, or 1·4 serum in the assay A much larger error is introduced by the serum binding of penicillin, as discussed in the preceding section

Tissue Inactivation In addition to the inactivation of penicillin by serum or plasma, there is strong evidence that it is bound and inactivated by tissues (15,16,70) In the case of penicillins F, G, and X, this inactivation proceeds so slowly in comparison with their excretion that 70 to 100 per cent is recovered in the urine. The local inactivation of penicillin may nevertheless be of major therapeutic significance, in that it may seriously limit the length of time for which penicillin remains in the tissues in effectively bactericidal concentrations.

Similar to the more rapid inactivation of penicillin K by plasma or serum, there is some evidence that it is inactivated (or bound) by the tissues to a greater extent than is penicillin G (15,16,70), for example The identity of this tissue inactivator with that present in

the serum, and the relationship between tissue binding and tissue inactivation have not been determined, however. There is strongly suggestive evidence (16,70) that penicillin K is bound by the liver to an even greater extent than penicillin G; if this reversible binding is followed by its inactivation, it may account for the major portion of the penicillin K "lost" after its injection

Whether because of its inactivation by plasma, tissues, or both, when penicillin K is injected in small doses, it disappears from the blood more rapidly than do penicillins F, G, or X similarly injected (7,8,14). Corresponding to this more rapid disappearance from the blood, a relatively small proportion (20–40 per cent) is recovered in the urine; the major portion is presumably inactivated *in vivo* After large injections, however, the blood levels of K fall off no more rapidly than those of the other penicillins; only a small proportion is then inactivated *in vivo*, and the urine recovery approaches normal values (38) It would therefore appear that, as is true of the inactivation of penicillin K by serum *in vitro*, the proportion of the drug inactivated varies inversely with its concentration This suggests that the inactivating mechanism specific for penicillin K may be present in relatively small concentration in the blood and tissues, and that it is readily saturated at high concentrations of the drug.

Relative Therapeutic Activity of Penicillins F, G, K, and X

PARADOXICALLY LOW THERAPEUTIC ACTIVITY OF PENICILLIN K

In the preceding sections, three pharmacologic factors have been discussed which tend to decrease the therapeutic efficacy of penicillin, and to decrease also the concentration available for diffusion into the tissue fluids. These factors are urinary excretion, binding by serum protein (and tissues), and inactivation by serum (and tissues). Penicillin K is peculiarly susceptible to the last two factors. It is bound by the plasma proteins to a much greater extent than the other penicillins, and is thus kept from diffusing into the tissues. Further, in low concentrations it is more rapidly inactivated by plasma; and there is considerable evidence that it is bound and inactivated by the tissues as well to a much greater extent than is penicillin F, G, or X (see above).

In consequence, although penicillin K is perhaps the most actively

TABLE IV

Relative Activity of Penicillins F, G, K, and X in Treatment of Experimental Infections with Streptococcus pyogenes (C-203), Diplococcus pneumoniae (type I), Borrelia recurrentis, and Treponema pallidum

Infecting organism	Ref. No	Size of inoculum	Penicillin treatment	Penicillin species F	G	K	X
				Relative therapeutic efficacy, based on curative dose in mg/Kg.			
Strep pyogenes	9	10^{-4}–10^{-7} cc	Oil-beeswax suspension 1–2, 7–8, and 24 hrs. after inoculation of mice	92	100	82	270
	10	10^{4}–$10^{4.6}$ M L.D	Aq soln, intramuscularly, 10 times at 3 hr intervals	50	100	9	260
D pneumoniae, type I	3 10	10^{3}–10^{5} M L D	Aq soln., intramuscularly (subcutaneously) 10 times at 3 hr. intervals, beginning 2 hrs after inoculation	83	100 100	19	300–500 160
Bor recurrentis	83	15 million organisms, intraperitoneally	One subcutaneous injection of aq soln., 22–24 hrs after inoculation	45	100	<30	15
	82a		One subcutaneous injection 2 days after intraperitoneal inoculation		100		15–20 (estimated from exptl. data as given)
T. pallidum, *in vivo*	79	10^{6}–10^{7} organisms, intratesticularly	24 intramuscular injections of aq soln. at 4 hr intervals, beginning 6 weeks after inoculation		100		
	80	10^{5}–10^{6} organisms, intradermally	24 intramuscular injections of aq. soln. at 4 hr. intervals, beginning 3 days after inoculation	36–64	100	15	63
	81	2×10^{3}, intradermally	4 intramuscular injections at daily intervals, beginning 4 days after inoculation	7–14 ±	100	11	13 ±

bactericidal of the four penicillins *in vitro* (6,10) (Fig 1), its therapeutic activity *in vivo* is extraordinarily low. In experimental infections with streptococci, pneumococci (7–10), or T. pallidum (79–81), penicillin K has been found to be only one-eighth to one-thirtieth as active as the other penicillins, measured in terms of the doses required to effect cure. These results, however, apply to a particular method of administration (multiple intramuscular injections of aqueous solutions). As is seen in Table IV, with other methods of administration penicillin K does not appear in so unfavorable a light. This is probably related to the fact that the degree to which penicillin K is bound or inactivated *in vivo* depends in part on its concentration in the body fluids. Indeed, if the experimental conditions were arranged so that the inactivation of penicillin K *in vivo* were reduced to negligible proportions, the very fact that it is more completely bound to the serum proteins and therefore excreted more slowly might make for a more sustained blood level, and might conceivably result in an enhanced rather than decreased therapeutic activity.

However undesirable penicillin K may be for systemic administration, it may be as useful as any of the other penicillins for topical use; and its relative therapeutic activity when given in large doses or when suspended in peanut oil and beeswax deserves further exploration

DIFFERENCES IN THERAPEUTIC ACTIVITY OF PENICILLINS F, G, AND X, AND THEIR PRACTICAL SIGNIFICANCE

As has been discussed in a preceding section, there are large and unpredictable differences in the relative bactericidal activities of penicillins F, G, and X *in vitro* Those differences have been indicated for four species of bacteria in Figure 1; and a similar twofold to fourfold difference in the direct bactericidal activity of penicillins F, G, and X has been reported for a large number of organisms (2–6,11,12). In addition, penicillins G and X differ significantly with respect to the magnitude and duration of the serum levels afforded by a given dose, penicillin X giving somewhat higher and more sustained values (3,5,7,8,14). Probably in consequence of these two factors, as much as a fivefold difference has been observed in the therapeutic activity of penicillins F, G, and X in a number of ex-

perimental infections (3,7–10,79–84). Some of these data have been summarized in Table IV.

At first sight, it would seem highly advantageous in the treatment of a given infection to use that particular species of penicillin which is most effective against the particular organism Usually, however, this is probably of little moment, since one need only administer a larger amount of a less effective penicillin in order to achieve the same result The possibility nevertheless remains that in a few infections which are relatively resistant to treatment (e g, bacterial endocarditis), cure might perhaps be effected more rapidly, and in a larger proportion of cases, if one could use that species of penicillin which is most active against the particular organism Investigation of this possibility is not feasible at the present time. With current methods of production, penicillin G is so much easier to prepare in quantity than either penicillin F or X that the bulk of the penicillin commercially available is a refined product which contains more than 90 per cent penicillin G Nevertheless, given the fact that penicillin X is far more active than G against a number of organisms both *in vitro* and *in vivo*, it deserves clinical evaluation in infections difficult to cure with penicillin G, and in which the causative organism has been demonstrated to be more susceptible to penicillin X

What Is Best Method of Administering Penicillin?

Although the direct bactericidal action of penicillin may not be solely responsible for its therapeutic action *in vivo*, it is nevertheless of major and perhaps of primary importance Since the rate at which bacteria are killed by penicillin varies with its concentration, the concentration at the focus of infection should be sufficiently high to insure the fastest possible bactericidal effect. The magnitude of this maximally effective level of penicillin varies markedly among different organisms (see Table II), and the treatment schedule must be adjusted to the susceptibility of the particular organism. With some organisms, for example, certain strains of Strep. pyogenes, a concentration at the focus of infection of as little as 0 064 μg per cubic centimeter (0 1 unit) ensures the maximum possible rate of bactericidal action, and as little as 0 006 unit is definitely bactericidal; while with more resistant organisms such as Strep. faecalis, the

tissue level must be maintained at 4 to 6 μg per cubic centimeter for maximum efficacy.

Due consideration must be given to the fact that most of the bacteria are usually not in the blood stream but in the tissues, and that the plasma level is not necessarily a measure of the actual concentration at the focus of infection (*1*) There will be a concentration difference due to the reversible binding of at least half of the circulating penicillin by the plasma proteins (*2*) Since penicillin must diffuse from the blood into the tissues, its rapid urinary excretion and

TABLE V

Frequency at Which a Given Dose of Penicillin G in Aqueous Solution Must Be Injected in Order to Maintain a Desired Plasma Level (50)

				To maintain a plasma concentration of penicillin G in excess of						
Micrograms per cubic centimeter				0.1	0.2	0.5	1.0	2.0	5.0	10.0
Oxford units per cubic centimeter				0.16	0.32	0.8	1.6	3.2	8.0	16.0
Dosage per Kg		Total dose in average adult		Injections indicated in the first column should be repeated at the intervals, in hours, indicated below						
Mg	Units	Mg	Units							
10	16,700	720	1,200,000	8.0	6.0	4.5	3.5	3.0	2.0	1.2
3	5,000	216	360,000	5.0	4.0	3.0	2.0	1.6	0.6	—
1.5	2,500	108	180,000	3.0	2.5	1.7	1.2	0.8	—	—
0.6	1,000	43	72,000	2.0	1.5	0.8	—	—	—	—
0.3	500	22	36,000	1.6	0.9	—	—	—	—	—
0.15	250	11	18,000	0.9	—	—	—	—	—	—

1 mg penicillin G = 1000 micrograms = 1667 Oxford units.

disappearance from the blood effectively limits the length of time for which penicillin is available for distribution; indeed, unless the injection is repeated before the concentration of diffusible penicillin in the plasma falls below that already attained in the tissue fluids, the direction of flow will probably be reversed (*3*) The indeterminate degree to which penicillin is inactivated or bound by the tissues themselves is yet another factor which may limit the concentration of penicillin free to act on the organism at the focus of infection At foci of infection which are poorly supplied by blood, the diffusion of penicillin into the area is so slow that this local inactivation may become an important factor. Under such circumstances, even if the plasma concentration were sustained at high levels for long periods, the concentration at the focus of infection

might nevertheless remain a small fraction of that in the circulating blood. (*4*) A final complication is introduced by the paradoxic fact that certain organisms, fortunately few in number, are killed much more slowly at high concentrations of penicillin than they are at a lower optimum concentration.

Not only must an effective concentration of penicillin be provided at the focus of infection, but no matter what the schedule of treatment, it must be maintained for a long enough aggregate period of time to achieve sterilization and cure. This time factor is determined by at least two variables (*1*) the rate at which the particular organism can be killed by penicillin, and (*2*) the number of organisms to be killed. Both of these factors vary within wide limits (see Table II on page 112); the minimum time within which it is possible to effect cure will vary accordingly, from the few hours which suffices in most gonococcic infections, to the minimum period of 4 days to 2 weeks required in syphilitic infection. The importance of the initial number of organisms for the ease with which an infection can be cured by penicillin has been clearly demonstrated in experimental rabbit syphilis (85)

CONTINUOUSLY MAINTAINED LEVELS VERSUS INTERMITTENT TREATMENT

The question is frequently raised as to whether it is necessary to *maintain* effectively bactericidal concentrations of penicillin in the tissues by repeating the injections at short intervals, or whether the drug can be given so infrequently that in the interval between injections the blood and tissue levels fall below those which are bactericidal *in vitro*.

Figure 12 is a diagrammatic representation of the tissue penicillin concentration after its intramuscular injection in aqueous solution, considered in relation to its bactericidal action. As long as the concentration is higher than that which is maximally effective (*A*), the bacteria are being killed at the fastest possible rate (except for those organisms which are killed at a paradoxically slower rate by penicillin concentrations in excss of an optimal level). At concentrations less than this maximally effective level (*A*), but greater than the minimum concentration which is demonstrably bactericidal *in vitro* (*B*), the bacteria are still being killed, but at a reduced rate.

Even after the penicillin level falls below this partially effective range, into a range of concentration which permits the multiplication of the organisms *in vitro*, the organisms do not immediately grow out. (*1*) In the first place, concentrations which are ineffective *in vitro* may nevertheless be effective *in vivo*, since organisms damaged but not killed by penicillin may have been rendered more sus-

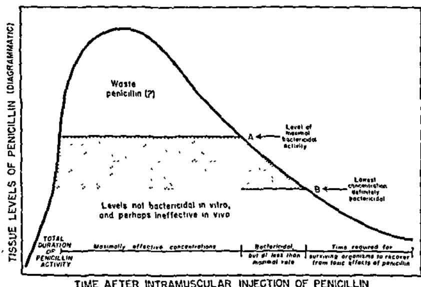


Fig 12 Diagrammatic presentation of tissue levels of penicillin after intramuscular injection of aqueous solution, in relation to duration of bactericidal effect (78).

ceptible to the body's natural defense mechanisms. (*2*) More important, if bacteria are exposed to penicillin, and the drug is then removed before all the organisms have been killed, the survivors do not immediately begin to multiply at their normal rate. Instead, there is a demonstrable "lag period" which may last for hours, during which the organisms are presumably recovering from the toxic effects of the drug, and during which they would be susceptible to the normal cellular and humoral defense mechanisms of the body. Because of this "recovery period," the blood and tissue penicillin levels may be permitted to fall below concentrations which are effectively bactericidal *in vitro* without prejudicing the outcome of treatment, pro-

vided only that this penicillin-free interval is not so long as to permit the recovery and significant remultiplication of the surviving organisms.

The danger that an infection may become re-established when there is a too long penicillin-free interval between injections is clearly greater if the organism is one capable of rapid multiplication Thus, in experimental pneumococcic infections in mice, in which the organisms multiply rapidly, a dosage which was regularly curative when given every three hours for 4 doses, was wholly ineffective when given every 9 hours (78). Apparently, the organisms grew out in sufficient numbers in the interval between injections to counteract the effect of otherwise curative doses, and the animals died. The mice could be cured even on the 9 hour schedule if the dosage per injection was increased threefold The larger dose was effective not because of the higher penicillin levels it provided, but because bactericidal levels were then maintained for a longer time, and the penicillin-free interval during which the surviving organisms could multiply and re-establish the infection was correspondingly reduced.

In marked contrast to pneumococci, there are several collateral lines of evidence that in rabbit and human syphilis the treponemata multiply only slowly, the division time in rabbits having been estimated to be on the order of 30 hours (85,88). Probably because of this, 16 injections of penicillin in aqueous solution (250 units/Kg.) each of which provided measurable serum levels for only $1^1/_2$–2 hours were therapeutically effective whether administered at 4, 8 to 16, or even 24 hour intervals On the 24 hour schedule, when the serum contained no demonstrable penicillin (<0 03 unit/cc.) for more than 22 hours out of the 24, there was so little multiplication in the interval between injections as to have only slight effect on the outcome of treatment (62). However, with an even longer interval between injections, the previously effective dosages were no longer curative Injections of 16,000 units per kilogram failed to cure any of the animals if given at intervals of, for example, 3 days or 1 week (86) Even the slow growing T. pallidum multiplied sufficiently in that long penicillin-free interval to counteract the effect of treatment In order to achieve cure in animals injected twice weekly or weekly it was necessary, either to increase the size of each injection markedly, or to use penicillin suspended in oil and beeswax (61) Both procedures served to prolong the time for

which penicillin was present in effective concentrations, and to reduce correspondingly the penicillin-free interval.

In summary, the fastest possible treatment with penicillin probably involves the maintenance of a maximally effective level at the foci of infection. However, treatment may be discontinuous (*1*) because penicillin may persist in the tissues for longer periods of time than in the blood, (*2*) because less penicillin may be necessary to kill bacteria *in vivo* than is necessary *in vitro*; and (*3*) because a significant time elapses before bacteria recover from its toxic effects sufficiently to resume multiplication at their normal rate The serum concentrations may be permitted to fall below the effectively bactericidal concentration for significant periods of time without necessarily prejudicing the outcome of treatment; however, the total time required to effect cure may thereby be prolonged

AQUEOUS SOLUTIONS VS PEANUT OIL–BEESWAX SUSPENSIONS

The use of penicillin suspended in oil and beeswax permits the maintenance of effectively bactericidal levels in the blood and tissues for long periods of time Injections need then be given only every 12, 24, or even 48 hours (Figs. 7,8,9), and treatment with penicillin on an ambulatory basis becomes a practicable procedure There are, however, definite if minor disadvantages to its use: pain at the site of injection, a somewhat more exacting technical procedure, a much greater tendency to sensitization reactions, and the fact that with highly resistant organisms it may not be possible to provide the maximally effective concentrations of 2 to 10 μg per cubic centimeter

The advantage in being able to treat some patients on an ambulatory, nonhospitalized basis, and the desirability of not disturbing a sick patient with unnecessary frequent injections, require no elaboration To a certain extent, these ends can be accomplished even with aqueous solutions by administering much larger doses. Thus, a single injection of 10 mg per kilogram representing a total of approximately 1,200,000 units in the average adult, will provide a measurable level in the blood for an average period of 8 to 12 hours (Fig 6) Since it is not essential that effectively bactericidal concentrations be maintained uninterruptedly, in most infections doses

of this order of magnitude could be given once every 12 hours. The complication introduced by organisms which are killed more slowly at high concentrations of penicillin than they are at lower optimal concentrations has already been considered (page 114).

Remark

This article was written during the summer of 1947. A large amount of important material has since appeared which the author regrets it was not possible to include.

References

1. Committee of Medical Research, O.S.R.D., Washington, and the Medical Research Council, London: Chemistry of Penicillin. Science *102*, 627, 1945.
2. Veldee, M. V., Herwick, R. P., and Coghill, R. D.: Recommendations of the International Conference on Penicillin. Science *101*, 42, 1945.
3. Welch, H., Putnam, L. E., Randall, W. A., and Herwick, R. P.: Penicillin X. Successful treatment of gonorrhea in a single intramuscular injection. J. A. M. A. *126*, 1024, 1944.
4. Libby, R. L., and Holmberg, N. L.: Activity of penicillins G and X *in vitro*. Science *102*, 303, 1945.
5. Ory, E. M., Meads, M., and Finland, M.: Penicillin X: Comparison with penicillin G with respect to sensitivity of pathogenic organisms and serum levels. J. A. M. A. *129*, 257, 1945.
6. Eagle, H.: The relative activity of penicillins F, G, K, and X against spirochetes and streptococci in vitro. J. Bact. *52*, 81, 1946.
7. Eagle, H., and Musselman, A.: The low therapeutic activity of penicillin K relative to that of penicillins F, G, and X, and its pharmacologic basis. Science *103*, 618, 1946.
8. Coghill, R. D., Osterberg, A. E., and Hazel, G. R.: The relative effectiveness of penicillins, G, K, and X. Science *103*, 709, 1946.
9. Hobby, G. L., Burkhart, B., and Hyman, B.: Chemotherapeutic action of various forms of penicillin on hemolytic streptococcal infections in mice. Proc. Soc. Exper. Biol. & Med. *63*, 296, 1946.
10. Eagle, H.: The therapeutic activity of penicillins F, G, K, and X in experimental infections with pneumococcus type I and Streptococcus pyogenes. J. Exper. Med. *85*, 175, 1947.
11. Welch, H., and Randall, W. A.: Sensitivity of Eberthella typhosa to penicillin fractions G and X. J. Lab. & Clin. Med. *32*, 193, 1947.
12. Nell, E. E., and Hill, J. H.: Comparison of the in vitro antigonococcal actions of penicillin G, F, K, and X. Am. J. Syph., Gonor. & Ven. Dis. *31*, 14, 1947.
13. Eagle, H.: The inactivation of penicillins F, G, K, and X by human and [illegible] J. Exper. Med. *85*, 141, 1947.
14. E[illegible] X

15 Richardson, A. P., Miller, I., Schumacher, C, Jambor, W., Pansy, F., and Lapedes, D.: Physiological disposition of penicillins G and K in dogs. Proc. Soc Exper. Biol & Med. *63*, 514, 1946.

16 Clowes, G. H A, and Keltch, A. K: On comparative results obtained with crystalline penicillins K, G, F, and X when exposed in the Warburg apparatus to the action of liver and other mammalian tissues, blood and blood fractions, and bacteria Supplementary report on the comparative results obtained with crystalline penicillins, particularly G and K, when exposed in the Warburg apparatus to the action of normal and preheated rat liver slices, homogenized liver, and bacteria; also normal liver with enzyme-suppressing chemical agents Reports at Meeting of Antibiotics Study Section of the National Institute of Health, Jan. 31–Feb. 1. 1947, Washington, D C.

17. Tompsett, R., Shultz, S, and McDermott, W.· The relation of protein binding to the pharmacology and antibacterial activity of penicillins X, G, Dihydro F, and K J Bact *53*, 581, 1947

18 Eagle, H · The kinetics of the bactericidal action of penicillin (streptococci, staphylococci, pneumococci and T. pallidum). I The effect of penicillin concentration In preparation

19 Hobby, G L, Meyer, K, and Chaffee, E · Observations on the mechanism of action of penicillin Proc Soc. Exper. Biol & Med *60*, 281, 1942.

20 Demerec, M Production of staphylococcus strains resistant to various concentrations of penicillin Proc Nat Acad Sc. *31*, 16, 1945

21 Eagle, H A paradoxical zone reaction in the bactericidal action of penicillin In preparation

22 Bondi, A, and Dietz, C C Penicillin-resistant staphylococci Proc. Soc Exper Biol. & Med *60*, 55, 1945

23 Hobby, G L, and Dawson, M H · Bacteriostatic action of penicillin on hemolytic streptococci *in vitro* Proc Soc Exper. Biol & Med *56*, 178, 1944

24 Gots, J S.: Production of extracellular penicillin-inactivating substances associated with penicillin resistance in Staphylococcus aureus Soc. Exper Biol. & Med *60*, 165, 1945

25 Watson, R F.: Sensitivity of various serologic groups of streptococci to penicillin Proc Soc Exper Biol & Med *57*, 65, 1944

26 Bondi, A, and Dietz, C C: Relationship of penicillinase to the action of penicillin Proc. Soc Exper Biol & Med *56*, 135, 1944

27 Bondi, A, and Dietz, C C Bacterial pencillinase· Production, nature, and significance. J Bact *51*, 125, 1946

28 Hobby, G L, and Dawson, M H · Effect of rate of growth of bacteria on action of penicillin Proc Soc Exper Biol & Med *56*, 181, 1944.

29 Bigger, J W. Treatment of staphylococcal infections with penicillin. Lancet *2*, 497, 1944.

30 Eagle, H, and Musselman, A D The spirochetICidal action of penicillin *in vitro* and its temperature coefficient J Exper Med *80*, 493, 1944.

31 Hobby, G L, Meyer, K., and Chaffee, E.· Observations on the mechanism of action of penicillin Proc Soc Exper. Biol & Med. *60*, 281, 1942.

32 Hoyt, R E, Pratt, O B, and Levine, M G · *The activity of penicillin at* temperatures above 37° C J Lab & Clin Med *30*, 736, 1945.
33 Lee, S W, and Foley, E J Effect of temperature on the action of penicillin *in vitro* Proc Soc Exper Biol & Med, *60*, 133, 1945
34. Eagle, H, Magnuson, H J, and Fleischman, R The effect of hyperpyrexia on the therapeutic efficacy of penicillin in experimental syphilis Am J Syph, Gonor & Ven Dis *31*, 239, 1947
35 Moore, J. E, Mahoney, J F, Schwartz, W, Sternberg, T., and Wood, W. B, Jr The treatment of early syphilis with penicillin A preliminary report of 1418 cases J A M A *126*, 67, 1944
36 The status of penicillin in the treatment of syphilis. A statement published under the joint auspices of the Syphilis Study Section of the National Institute of Health, U S Public Health Service, and the Council of Pharmacy and Chemistry, American Medical Association J A. M. A, in *press*
37 Luria, S E Recent advances in bacterial genetics Bact Rev *11*, 1, 1947
38 Eagle, H · Unpublished data
39 Bahn, J M, Ackerman, H, and Carpenter, C M Development *in vitro* of penicillin-resistant strains of the gonococcus Proc Soc Exper. Biol & Med *58*, 21, 1945
40 Miller, C P, and Bohnhoff, M Studies on the action of penicillin IV tures of staphylococci, pneumococci and streptococci Proc Soc Exper Biol & Med *53*, 33, 1943
41 McKee, C M, and Houck, C L Induced resistance to penicillin of cultures of staphylococci, pneumococci and streptococci Proc Soc Exper Biol & Med *53*, 33, 1943
42 Dowling, H F, Hirsh, H L, and O'Neil, C B Studies on bacteria developing resistance to penicillin fractions X and G *in vitro* and in patients *under treatment for bacterial endocarditis* J *Clin Investigation 25*, 665, 1946
43 Miller, C P, and Bohnhoff, M Studies on the action of penicillin V Virulence of penicillin-resistant strains of Meningococcus Proc Soc Exper Biol & Med *60*, 356, 1945
44 Youmans, G P, Williston, E H, and Simon, M Production of small colony variants of Staphylococcus aureus by the action of penicillin Proc Soc Exper Biol & Med *58*, 56, 1945
45 Rake, G W, McKee, C M, Hamre, D M, and Houck, C L Studies on penicillin II Observations on therapeutic activity and toxicity J Immunol *48*, 271, 1944
46 Spink, W W, and Ferris, V Penicillin inhibitor from staphylococci which have developed resistance to penicillin in the human body Proc Soc Exper Biol & Med *59*, 118, 1945
47 Spink, W W, Hall, W H, and Ferris, V Clinical significance of staphylococci with natural or acquired resistance to the sulfonamides and to penicillin J A M A *128*, 555, 1945
48 Schmidt, L H, and Sesler, C L Development of resistance to penicillin by pneumococci Proc Soc Exper Biol & Med *52* 353, 1943

49 Kirby, W. M M Extraction of a highly potent penicillin inactivator from penicillin resistant staphylococci Science 99, 452, 1944
50 Tucker, H A, and Eagle, H.: The serum concentration of penicillin G in man following its intramuscular injection in aqueous solution and in peanut oil–beeswax suspension. Am J Med. 4, 343, 1948
50a Eagle, H, Fleischman, R, and Musselman, A: The serum concentration of penicillin G in mice, rabbits and man, after its intramuscular injection in aqueous solution J. Bact, Jan, 1949
51 Rammelkamp, C H, and Bradley, S W Excretion of penicillin in man Proc Soc Exper Biol & Med 53, 30, 1943
52 Rantz, L. A, and Kirby, W M M: Absorption and excretion of penicillin following continuous intravenous and subcutaneous administration J Clin Investigation 23, 789, 1944
53 Jensen, K A, Møller, K O, and Overgaard, K S: Studies on the excretion of penicillin through the kidneys and the mechanism of this process Acta pharmacol et toxocolog 1, 184, 1945
54 Eagle, H, and Newman, E V: The renal clearance of penicillins F, G, K and X in rabbits and man J. Clin. Investigation 26, 903, 1947
55 Chow, B F, and McKee, C M Interaction between crystalline penicillin and human plasma proteins Science 101, 67, 1945
56 Beyer, K H, Peters, L, Woodward, R, and Verwey, W. F Enhancement of the physiological economy of penicillin in dogs by the simultaneous administration of para-aminohippuric acid II. J Pharmacol. & Exper. Therap. 82, 310, 1944
57 Beyer, K H, Flippin, H, Verwey, W. F, and Woodward, R The effect of para-aminohippuric acid on plasma concentration of penicillin in man J A M A 126, 1007, 1944
58 Beyer, K H, Miller, A Russo, K, Patch, H F, and Verwey, W F: The inhibitory effect of caronamide on the renal elimination of penicillin Am J Physiol 149, 355, 1947
59 Romansky, M J, and Rittman, G E Penicillin Prolonged action in beeswax–peanut oil mixture Single injection treatment of gonorrhea Bull U S Army Med Dept No 81, 43, 1944
60 Romansky, M J Recent data on crystalline penicillin in peanut oil and beeswax Report at Meeting of Antiobiotics Study Section of the National Institute of Health, Jan 31–Feb 1, 1947, Washington, D C
61 Eagle, H, Magnuson, H J, and Fleischman, R Observations on the therapeutic efficacy in experimental syphilis of calcium penicillin in oil and beeswax and their bearing on its use in man Am J Syph 31, 246, 1947
62 Eagle, H, Magnuson, H J, and Fleischman, R The effect of the method of administration on the therapeutic efficacy of sodium penicillin in experimental syphilis Bull Johns Hopkins Hosp 79, 168, 1946
63 Seager, L D Blood levels of penicillin after oral administration with various antacids Science 103, 353, 1946
64 Finland, M, Meads, M, and Ory, E M Oral penicillin, J A M. A 129, 315, 1945
65 McDermott, W, Bunn, P A, Benoit, M, DuBois, R, and Reynolds, M. E

The absorption, excretion and destruction of orally administered penicillin J Clin Investigation *25*, 190, 1946
66 Cutting, W. C, Halpern, R. M, Sultan, E H, Armstrong, C D, and Collins, C L. Administration of penicillin by mouth with results in the treatment of gonorrhea. J. A. M A *129*, 425, 1945.
67. Meakins, J C, Smith, F, and Gold, M. F · A comparative study of some commercial preparations of oral penicillin Canad M. A. J. *55*, 97, 1946
68 Gyorgy, P, Evans, K. W, Rose, E. K, Perlingiero, J. G, and Elias, W F.. Peroral penicillin. Pennsylvania M. J. *49*, 409, 1946
69 Taplin, G. V.: Administration of micronized therapeutic agents by inhalation or topical application Science *105*, 502, 1947.
70 Tepperman, J, Rakieten, N, Valley, G, and Lyon, E W.· Inactivation of penicillins G and K by liver and kidney. Science *105*, 18, 1947
71 *Rammelkamp, C. H., and Keefer, C S · The absorption, excretion and distribution* of penicillin J Clin Investigation *22*, 425, 1943
72 McDermott, W., and Nelson, R A The transfer of penicillin into the cerebrospinal fluid following parenteral administration Am J Syph *29*, 403, 1945
73 Cooke, J V, and Goldring, D The concentrations of penicillin in various *body fluids during penicillin therapy. J A. M A 127, 80, 1945*
74 Balboni, V G, Shapiro, I. M, and Kydd, D M The pentration of penicillin into joint fluid following intramuscular administration Am J. M Sc *210*, 588, 1944
75 Greene, H J, and Hobby, G L Transmission of penicillin through human placenta Proc Soc. Exper Biol & Med *57*, 282, 1944
76 Struble, G C, and Bellows, J G Studies on the destruction of penicillin in the eye and its chemical application J A M A *125*, 685, 1944.
77 Bigger, J W : Inactivation of penicillin by serum Lancet *2*, 400, 1944
78 Eagle, H Speculations as to the therapeutic significance of the blood penicillin *level Ann Internal Med 28, 260, 1948*
79 Arnold, R C, Boak, R A, Carpenter, C M, Chesney, A. M, Fleming, W L, Gueft, Boris, Mahoney, J F, and Rosahn, *P. D · A joint report* on a cooperative investigation of the efficacy of species of penicillin in the treatment of experimental syphilis Am J Syph, Gonor & Ven Dis *31*, 469, 1947.
80 Rake, G W, Dunham, W B, and Donovick, R Evaluation of antibiotics by the prevention of experimental syphilis J. Infect Dis *81*, 122, 1947
81 Eagle, H *The relative antisyphilitic activity of penicillins F, G, K, and* X and of bacitracin, based on the amounts required to abort early syphilitic infection in rabbits J Bact *55*, 341, 1948
82 Cutting, W C, Luduena, F P, Fiese, M, Elliott, H W, and Field, J, II Distribution and fate of penicillin in the body J Pharmacol & Exper Therap *85*, 36, 1945
82a Williamson, J, and Lourie, E M *Therapeutic action of different penicillins* on Spirocheta recurrentis infections in mice. Brit M J *1*, 828, 1946
83 Schnitzer, R J, and Farr, H C: Activity of penicillins F, G, K, and X

as determined with the Borrelia assay method *in vivo*. Report at Meeting of Antibiotics Study Section of the National Institute of Health, Jan 31–Feb 1, 1947, Washington, D. C

84 Powell, H M, and Clowes, G H A.: On the comparative results obtained with crystalline penicillins K and G when injected simultaneously and at various time periods before and after the injection of 1000 lethal doses of Streptococcus hemolyticus (C-203) into the peritoneal cavities of standard mice. Reported at Meeting of Antibiotics Study Section of the National Institute of Health, Jan 31–Feb 1, 1947, Washington, D C.

85 Magnuson, H. J, Eagle, H, and Fleischman, R: The minimal infectious inoculum of Spirochaeta pallida (nichols strain), and a consideration of its rate of multiplication in vivo Am J Syph, Gonor. and Ven. Dis. *32*, 1, 1948.

86 Mahoney, J. F, and Arnold, R. C Personal communication.

87 Graessle, O E, and Frost, B M: Induced *in vitro* resistance of staphylococci to streptomycin and penicillin Proc. Soc Exper. Biol. & Med. *63*, 171, 1946

88. Cumberland, M C, and Turner, T. B: The rate of multiplication of Treponema pallidum in normal and immune rabbits Am J. Syph, Gonor and Ven Dis In press

Streptomycin: Development and Status of Its Use in the Treatment of Tuberculosis

H. Corwin Hinshaw
Division of Medicine, Mayo Clinic

AND

William H Feldman
Division of Experimental Medicine, Mayo Foundation, Rochester, Minnesota

The renaissance of specific drug therapy of infectious diseases began when Domagk (7) in 1935 announced that a sulfonamide compound—prontosil—was a highly effective therapeutic agent against infections due to hemolytic streptococci Ehrlich's announcement of arsphenamine, which had ushered modern chemotherapy in so auspiciously, had been followed by some advances in the chemotherapy of certain spirochetal and protozoal diseases but successful attack on the bacterial infections was not accomplished until the advent of the sulfonamides

Tuberculosis was among the infectious diseases that had resisted successful attack by specific therapeutic substances up to the time when prontosil was announced * The quest for a specific drug that would effectively combat this most important infectious disease had been pursued with varying intensity by many workers ever since the causative agent had been definitely establishd by Koch (38) in 1882. This phase of the problem has been reviewed by Feldman (11) and by Hart (25) Suffice it to say that while many substances were found that were capable of marked antagonism against tubercle bacilli in the test tube, none were found that would successfully suppress a fully virulent progressive tuberculous infection The success of the sulfonamides as therapeutic weapons against a considerable number of other infectious agents encouraged the belief that these

* A sketch of the historical development of chemotherapy in general has recently been contributed by Lowrie (39)

new drugs offered an exceptional opportunity to reopen the problem of specific drug therapy in tuberculosis.

In 1938, Rich and Follis (49) announced the results of three experiments in which guinea pigs infected with tubercle bacilli were treated with sulfanilamide. The results indicated that sulfanilamide could, under the conditions imposed, retard the rate of development of tuberculous lesions in experimental animals. While the drug failed to suppress completely, or to eliminate, the infective bacilli, the morphologic aspects of the experimental disease were favorably influenced. Although the deterrent effects were of limited degree, they were definite and the results were significant. However, none of the sulfonamide derivatives have found clinical application in the treatment of tuberculosis.

Promin, a derivative of diaminodiphenyl sulfone, was the first chemotherapeutic substance to demonstrate the remarkable ability of arresting tuberculosis in guinea pigs, produced by inoculation with virulent strains of Mycobacterium tuberculosis hominis (17).

At approximately the same time, Rist, Bloch, and Hamon (50) independently demonstrated that 4,4'-diaminodiphenyl sulfone exerted a definite inhibitory effect on infections in rabbits and guinea pigs due to avian tubercle bacilli.

This compound and related "sulfone" drugs have been studied extensively in several laboratories since 1940 in the hope that clinical applications might be developed. Unfortunately, these sulfone drugs are relatively more toxic for human beings than they are for guinea pigs, a fact which has thus far sharply limited their clinical use. Nevertheless, the discovery that virulent tubercle bacilli, developing in an extremely susceptible host, could be inhibited sufficiently to permit rapid healing of lesions, led to frequent expressions of optimism regarding the future of antibacterial therapy in tuberculosis.

The status of the sulfone compounds in experimental and in clinical tuberculosis has been reviewed and summarized recently by Feldman (10).

Since the development of promin, considerable progress has been made in the specific chemotherapy of tuberculosis (9–11). The results of the many investigations have shown that tuberculosis is no longer refractory to specific drug therapy and that the many supposed barriers to direct attack on the bacillus of tuberculosis do not exist. As new antibacterial drugs appeared, they were tested against experimental tuberculosis and compared with the effective sulfone

preparations. Consequently, when streptomycin was announced by Schatz, Bugie, and Waksman (52) as an antibiotic substance against gram-negative bacilli, it was tested in our laboratory against experimental tuberculosis and later against clinical tuberculosis.

Antibiotics as Antituberculosis Agents

The existence of substances of microbial origin capable of antibacterial effects was recorded 70 years ago by Pasteur and Joubert (47). They noted that certain air-borne bacteria inhibited the growth of the anthrax bacillus The medical significance of this phenomenon was apparent to Pasteur and Joubert, who wrote: "These facts justify the highest hopes for therapeutics." Ever since they made this observation, there have been many attempts to make direct or indirect use of microbial antagonists in the treatment of infectious diseases. (An admirable review on the use of micro-organisms in therapeutics, including tuberculosis, has been contributed by Florey (22).)

When the age-old importance of tuberculosis is considered, it is not surprising to find that 44 years before Fleming's first report on penicillin, an attempt had been made to obtain therapeutic effects in clinical tuberculosis by antagonistic substances of bacterial origin (4). A review of the literature pertaining to the search for substances of microbial origin that would exert antagonistic influences against tubercle bacilli discloses that many such agents have been found which are effective on cultures of tubercle bacilli (11,25). However, because of the difficulties inherent in the production, extraction, and purification of antibiotics, none of these have been subjected to tests adequate to determine their value as therapeutic agents. In the majority of instances, evidence of antibacterial effects against the tubercle bacillus was limited to the results of *in vitro* testing only. Very few of the reported substances have received a crucial test of *in vivo* application in experimental tuberculosis of animals. An outstanding exception is to be found in the case of streptomycin. This antibiotic agent has successfully passed, in the United States, the crucial test in animals and in human beings, and it is now being produced in quantities sufficient for commercial distribution and clinical use.

Nature of Streptomycin

Description. Streptomycin is a highly potent antibiotic agent discovered by Schatz, Bugie, and Waksman (52). It was found as the result of a search for products capable of antagonistic effects against gram-negative bacilli. Streptomycin is derived from a sporulating and aerial-mycelium-forming species of actinomycete, Streptomyces griseus The antibiotic can be produced in both natural and synthetic mediums, in submerged as well as surface culture. Temperatures for maximal production of streptomycin lie between 25 and 30 C Chemically, streptomycin is an organic base, soluble in water but insoluble in organic solvents. The approximate chemical formula of streptomycin hydrochloride is given as $C_{21}H_{39}N_7O_{12}\,3HCl$ by Waksman (56).

Stability Streptomycin, unlike penicillin, is markedly stable Concentrations of the drug stored at 10 C. at *p*H 6, 7, and 8 were found to be stable for a period of 3 months (46) We have frequently observed that solutions may be kept at room temperature for several days or more without loss of potency. Furthermore, this antibiotic agent is not inactivated or destroyed by micro-organisms. The substance is, however, inactivated by cysteine

Unitage A unit of streptomycin was originally defined as an "S" unit, which was the amount of the antibiotic sufficient to inhibit the growth of a certain strain of Escherichia coli in 1 ml of nutrient broth For practical reasons, the "S" unit has been abandoned and quantities of streptomycin are expressed on a weight basis. One microgram is approximately equivalent to 1 "S" unit Thus, 1 Gm of pure streptomycin is equivalent to 1,000,000 "S" units

Range of Bacterial Activity In addition to tuberculosis, streptomycin has been found to be a useful therapeutic agent in several bacterial infections These infections include (*1*) tularemia, in the treatment of which streptomycin is highly specific (23), (*2*) certain types of bacterial endocarditis (33), (*3*) meningitis caused by Hemophilus influenzae (1), (*4*) the gram-negative contaminants of wounds (32); (*5*) bacteremia produced by Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Aerobacter aerogenes, and Pseudomonas aeruginosa (37); (*6*) certain infections of the urinary tract (28), (*7*) bronchiectasis (45); and (*8*) experimental bubonic plague (41).

Evidence at the present time indicates that streptomycin is of

questionable value in cases of typhoid fever, chronic brucellosis and Salmonella infections.

Recently Finch (21) reported on a small series of patients affected with acute brucellosis and treated with streptomycin The results were considered definitely encouraging.

The drug is considered ineffective in malaria, in all clostridial, rickettsial and viral infections, and in infections produced by fungi and by molds

Administration and Dosage. Streptomycin may be administered intramuscularly, subcutaneously, intrathecally, or by nebulization. In the treatment of experimental tuberculosis in guinea pigs we have always given the drug subcutaneously. Clinically, the drug is usually administered intramuscularly, although in meningeal infections intrathecal injections of the drug are usually given, in addition to intramuscular injections Infections of the upper part of the respiratory tract, including the pharynx and laryngeal region, and lesions of the trachea and larger bronchi are frequently treated by nebulization of the drug together with the usual intramuscular administration.

In our experience, the preferred dose of streptomycin in cases of tuberculosis has been from 1 0 to 2 0 Gm per day. Unquestionably, the lower dose is much preferable to the higher one, both as regards toxicity and expense, and undoubtedly this dose is adequate in some, if not in most, instances Whether it will be adequate for all circumstances has not been determined. The higher dosage levels were originally selected in the hope that this would prevent the occurrence of drug-fast strains of tubercle bacilli This has not been the case This reason for large dosage therefore no longer exists, and most investigators are using the smaller dosage schedule

It is difficult to make definite recommendations as to dosage, but we suggest that a dose of 1 0–2 0 Gm per day is reasonably safe and definitely within the therapeutic range Originally, the drug was administered in small doses at frequent intervals, but now it appears, as a result of studies on animals and patients, that the dosage may be administered in 1 or 2 doses per day.

Intramuscular injection is preferable, but subcutaneous injection is possible with highly purified streptomycin. Solutions for injection may be made up in a concentration of from 100 to 250 mg per milliliter, the diluent being pyrogen-free, triple-distilled water.

Special instructions for aerosol therapy have been described elsewhere (45), the usual dose being 500 mg. dissolved in 10 to 20 ml of isotonic saline solution. Cases of tuberculous meningitis require, in addition to the usual injections, intrathecal administration of 25 to 100 mg. of streptomycin each 24 to 72 hours.

Excretion. For estimating the concentration of streptomycin in the blood, spinal fluid, and other body fluids, the technic described by Herrell and Heilman (27) may be followed. Excretion of streptomycin is accomplished by the kidneys, but the drug is widely distributed throughout the tissues of the body except the brain. The drug has been demonstrated in most of the body fluids, including pleural and peritoneal exudates, aqueous and vitreous humors, and bile (26). Streptomycin may also be excreted in human milk (44) The drug also enters the fetal circulation (26). When given orally, little if any streptomycin is absorbed from the gastrointestinal tract. Streptomycin given orally has a marked suppressive effect on the bacterial flora of the bowel, since most of the drug given by this route is eliminated with the feces.

Toxicity Of first importance in considering any substance for clinical use is its toxicity. (Pharmacologic observations on streptomycin have been reported by Molitor, 43, and by McDermott, 40 While streptomycin in its present form* has some toxic properties, experiments on animals and clinical studies have demonstrated that among chemotherapeutic agents streptomycin has a reasonable margin of safety when serious types of disease are considered. When streptomycin is used clinically in a disease such as tuberculosis, in which treatment of relatively long duration may be indicated toxic manifestations are observed fairly often. The most frequent untoward reactions reported have been those referable to the eighth cranial nerve, in which the vestibular functions have frequently been definitely reduced. Rarely, auditory function is impaired when large doses have been administered for prolonged periods or when excretion of the drug is delayed by impaired renal function Other toxic manifestations that have been noted include erythematous rash, fever, eosinophilia, and abnormal urinary sediments. After intra-

*The appearance of dihydrostreptomycin since preparation of this chapter has permitted streptomycin therapy with larger doses and greater safety. However, all of the precautions advised in this chapter apply also to dihydrostreptomycin

muscular injection some, but not all, commercial preparations of streptomycin have produced considerable inflammatory reaction at the site of injection.

Of the various untoward effects of streptomycin therapy in human beings, those involving the eighth cranial nerve are the most frequently observed and are the cause of considerable discomfort (3). In cases requiring the prolonged use of streptomycin, the significance of possible damage to the eighth nerve should be recognized and the expected benefits to be derived from the treatment should be balanced against possible dysfunction that may result from its use.

The factors responsible for the predilection of streptomycin for the eighth cranial nerve are not known Whether the neurologic disturbances are due to impurities or to causes inherent in the drug is unsettled. However, Molitor (43), after reviewing the questions, stated recently that the "findings strongly suggest that the neurotoxic effects of streptomycin are due, at least in part, to an impurity." Thus the possibility exists that neurotoxic reactions will be reduced as a more complete purification of the drug is achieved.

So far, there is no evidence of serious damage to the liver after streptomycin therapy, and renal damage is to be feared only if there is preexisting renal disease

Finally, while the possible toxic effects of streptomycin must be recognized, evidence which has accumulated from extensive clinical trials indicates that in serious or potentially serious tuberculous infections the question of toxicity does not constitute a valid contraindication to its use. This drug, like many others, should be used with discretion in doses designed to exert maximal efficacy and minimal toxic reaction

Effectiveness of Streptomycin against Tubercle Bacilli *in Vitro*

The *in vitro* sensitivity to streptomycin of tubercle bacilli of the human type was first observed by Schatz, Bugie, and Waksman (51,52) In this respect, streptomycin resembles other substances derived from growth of microorganisms (22,56) In studying a considerable number of bacteria belonging to several different genera, these workers noted that the human type of tubercle bacilli has a moderate sensitivity to streptomycin Both bacteriostatic and bactericidal effect were recorded

Youmans (58) also studied the *effects of streptomycin in vitro* on the human type of tubercle bacilli. In addition, he studied the relation of the number of bacteria to the bacteriostatic action of streptomycin, and the influence of plasma on the action of the drug *in vitro*. He made use of 5 fully virulent strains of tubercle bacilli. Youmans *found that the minimal concentration of streptomycin necessary for* bacteriostatic action is somewhere between 0 095 and 0.78 μg. per milliliter. The degree of sensitivity to streptomycin was comparable for all 5 strains studied. Further studies by Youmans and Karlson (59) on 131 strains of tubercle bacilli of the human type showed that the growth of 90 per cent of the strains was inhibited by 1 56 μg. or less of streptomycin per milliliter. None of the patients from whom the respective strains were obtained were receiving streptomycin when the tubercle bacilli were isolated. This was in approximate agreement with the results of Schatz and Waksman (51). To obtain a bactericidal effect *in vitro*, Youmans (58) found that a concentration of streptomycin in excess of 50 μg. per milliliter was necessary Neither the number of bacteria nor the presence of plasma had any significant effect on the bacteriostatic action of the drug

In vitro studies on the sensitivity to streptomycin of strains of bovine tubercle bacilli indicate that the bovine type of the organism has a sensitivity comparable to that of the human type of bacillus. *In vitro* studies of strains of avian tubercle bacilli have shown that this variety of the tubercle bacillus may have a greater resistance to streptomycin than is true of the human and the bovine types of the organism.

Effectiveness of Streptomycin in Experimental Tuberculosis

Our studies on the effect of streptomycin in cases of experimental tuberculosis began in April, 1944, a few months after the announcement of its discovery by Schatz, Bugie, and Waksman in January, 1944 * Studies on antibacterial treatment of experimental tuberculosis had been under way in our laboratory for several years and

* The streptomycin used in our first experiment was kindly supplied by Dr S A. Waksman Streptomycin used in our subsequent experiments was supplied through the courtesy of Drs J. M Carlisle and D F Robertson, Merck & Co., Inc., Rahway, N J

had demonstrated the feasibility of this approach The methods of study which had been developed during previous years permitted a roughly quantitative assay of the effectiveness of various drugs in the treatment of the experimentally produced disease. The results of our initial observations (13) clearly suggested the high potency of streptomycin

Our first results on streptomycin as a deterrent agent against tuberculosis in guinea pigs, although obtained from observations on a very small group of animals, were sufficiently conclusive to justify the further investigation of streptomycin as an antituberculosis drug During the past 4 years a considerable number of *in vivo* experiments on animals have been completed The results obtained from these studies indicated that the use of streptomycin is justified in selected cases of clinical tuberculosis

No attempt will be made here to describe in detail all of the experiments that we have conducted in order to establish the deterrent action of streptomycin in experimental tuberculous infections, a detailed report of our earlier studies having been published previously (15) However, because of the recent development of streptomycin therapy in tuberculosis and the interest in streptomycin as a practical approach to specific drug therapy in tuberculosis, it is considered advisable to provide the reader with a résumé of the experimental evidence on which subsequent clinical use of the drug has been based

First Experiment In our first experiment with streptomycin, the amount of the drug available, as mentioned previously, permitted treatment of only 4 guinea pigs Each animal was inoculated subcutaneously with 0 1 mg of our stock strain of tubercle bacilli, H37Rv Treatment of 2 of the guinea pigs was started immediately and continued daily until the end of the experiment Treatment of the other 2 animals was delayed for 2 weeks Streptomycin was given every 3 hours during the period from 9 a m to 9 p m The streptomycin available was exhausted on the fifty-fourth day after infection, and the 4 treated animals as well as 7 untreated animals which had been inoculated with tubercle bacilli at the same time as the treated animals were killed The results were striking The differences in the amounts of tuberculosis in the treated and untreated animals were impressive, and suggested that streptomycin is an antituberculosis agent of considerable potency *in vivo*

Second Experiment The results of the first experiment made a second experiment imperative The second experiment was essentially like the first, except that sufficient streptomycin was available so that a large number of animals could be used Each of 20 guinea pigs was inoculated subcutaneously

with 0 1 mg of tubercle bacilli, while 10 animals were not treated and served as controls

Treatment of 6 of the animals was started on the day of inoculation and treatment of 4 was started 2 weeks later As 1 animal in the treated group and 1 in the untreated group died prematurely, the final results were based on a total of 18 rather than 20 animals

The results of the second experiment were comparable in every respect to those observed in the first experiment. The disease in the untreated controls was widely disseminated and in most instances destructive In the treated animals the reverse was true; signs of disease were absent or barely detectable Furthermore, there were no significant differences in the effects of treatment in the group in which treatment was started on the day of infection and in the group in which the beginning of treatment was delayed

The results of the first two experiments left no doubt of the ability of streptomycin to suppress consistently and definitely a potentially lethal tuberculous infection in guinea pigs Additional studies demonstrated that, although the activities of the tubercle bacilli had been inhibited, some of the organisms remained viable. Very important, however, was the evidence that streptomycin was well tolerated by guinea pigs There were no recognizable evidences of toxicity of the drug. These findings justified a third and crucial experiment

Third Experiment. A detailed account of the experiment has been published previously (15) In this experiment 49 guinea pigs were used This was many more animals than had been utilized in the previous experiments with streptomycin In addition, the experimental conditions in the third experiment were more rigorous, in that treatment was delayed for 7 weeks after the animals had been infected Immediately prior to treatment a specimen of the liver for biopsy was obtained from each animal This was to provide information on the state of the infection prior to treatment so that the information obtained could be compared with the situation in the respective livers when the experiment was completed

All animals were inoculated with 0 001 mg of tubercle bacilli of human type, 42 days later all animals reacted positively to tuberculin Biopsies of the liver were done 48 days after infection Treatment was started on the forty-ninth day; 25 animals were treated with streptomycin; 24 were not treated [illegible] 6 mg of streptomycin [illegible] nent was continued for [illegible] ed 215 days after the animals had been infected.

There was a marked difference between the mortality rate of the treated group and that of the untreated group (Fig. 1). Although approximately 70 per cent of the controls died during the period of observation, only 2 (8 per cent) of those that were treated died

during this time. Necropsy revealed severe, widely disseminated tuberculosis in nearly all of the untreated controls. Among the animals that had been treated, evidence of tuberculosis was either absent or minimal (Fig. 2). As a matter of fact, 52 per cent of the animals that had been treated had no discernible tuberculosis either grossly or microscopically Although all animals were sensitive to tuberculin when treatment was begun, 9 of the animals (39 per

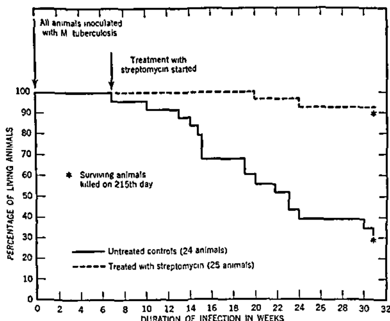
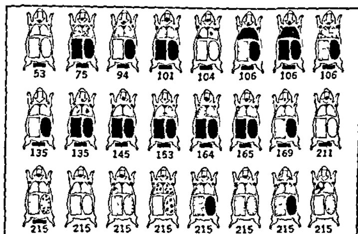


Fig. 1. Survival times of treated and untreated groups of guinea pigs (15)

cent) that had received streptomycin and were living failed to react to tuberculin at the time the experiment was concluded In 7 of these 9 animals, no residual virulent tubercle bacilli could be demonstrated in the spleens by subinoculation of normal guinea pigs.

The organs of predilection of all the animals were examined microscopically The histologic examination of the tissues of the animals that had received streptomycin provided pertinent information regarding the efficacy of the therapeutic procedure. In only 1 of these animals was a tuberculous lesion found in the liver, this was

CONTROLS



STREPTOMYCIN SERIES
TREATED AFTER 49 DAYS

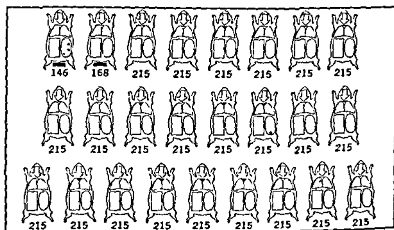


Fig 2 Amount of tuberculosis, noted grossly and recorded schematically, at time of necropsy in guinea pigs treated with streptomycin and in untreated controls The numerals represent the length of survival in days after the animals had been infected with tubercle bacilli A black bar above a numeral indicates that the animal died (15)

a small, atrophic nodule. In only 1 animal was a parenchymal lesion found in the lungs, the disease in this animal was represented by a

solitary, calcified nodule. The spleens of 14 of the animals were without microscopic evidence of tuberculosis Tuberculous changes were recognized in the spleens of 11 animals; however, in 7 the lesions were calcified (Fig 3), in 3 they were fibrotic (Fig 4), and in 1 they consisted of epithelioid or hard tubercles Further impressive evi-



Fig 3 Calcified nodule (×130) in the spleen of a tuberculous guinea pig after treatment with streptomycin for 166 days

dence of the therapeutic efficacy of streptomycin was the paucity of the disease in the axillary lymph nodes contiguous to the site of inoculation In only 2 of the treated animals was involvement of an axillary lymph node observed In no instances were residual signs of the disease found in the subcutaneous tissues at the site of injection over the sternum

The data obtained from our third experiment seem to substantiate certain definite conclusions regarding the therapeutic effects of

streptomycin in experimental tuberculosis. Among the more important conclusions are the following:

(1) Streptomycin, under the conditions imposed, was effective in resolving or suppressing experimental tuberculosis in guinea pigs established several weeks before treatment was started



Fig 4 Spleen of tuberculous guinea pig treated with streptomycin for 119 days The lesion (×130) is no longer characteristically tuberculous, having become a nonspecific fibrotic nodule (15)

(2) Although capable of undeniable deterrent effects in combating or preventing anatomic changes due to Mycobacterium tuberculosis, streptomycin in most instances exerted a suppressive rather than a sterilizing effect on the infective agent

(3) In more than a third of the treated animals a reversal of a previously demonstrated sensitivity to tuberculin occurred

(4) The unquestionable ability of streptomycin to reverse the potentially lethal course of inoculation tuberculosis in guinea pigs,

and the relatively low toxicity and corresponding safety of purified streptomycin satisfy the prerequisites of a chemotherapeutic agent worthy of a clinical trial.

Bacterial Strain Specificity. The observation having been made that streptomycin is effective in suppressing infections induced in guinea pigs by a laboratory stock strain of tubercle bacilli, it was advisable to determine if the drug is likewise effective against infections produced by recently isolated strains of tubercle bacilli. The clinical importance of such information is obvious.

In all, 10 studies were done The inoculums were prepared by using 7 strains of tubercle bacilli obtained by culture from gastric aspirations from 7 patients who had severe, progressing pulmonary tuberculosis In addition, inoculums were prepared from 2 strains of bovine type of tubercle bacilli, and, for comparison, the tenth inoculum was prepared from the laboratory stock strain H37Rv. For the tests, 10 groups of 14 guinea pigs each were used Each of the 14 guinea pigs in one group was inoculated subcutaneously with 0 1 mg. of one of these strains of tubercle bacilli, and 2 weeks later treatment with 6 mg of streptomycin daily, divided into 4 equal doses 6 hours apart, was started for 8 animals in each group of 14 Treatment was continued for 54 days. This experiment was terminated on the sixty-eighth day after infection (11,12)

Evidence that streptomycin had exerted a deterrent effect in each of the 10 groups of animals was conspicuous The marked dissimilarity of the gross appearance of the untreated controls and of the treated animals in all groups at the time of necropsy provided convincing evidence of the effect of treatment. Proof of the marked virulence of the respective strains was unequivocal In most instances the disease in the untreated controls had extensively affected the spleen, liver, and lungs In addition, there was striking tuberculous involvement of the lymph nodes contiguous to the site of inoculation The initial focus of infection in the tissues at the site of inoculation had persisted and progressed

Among the animals in the respective treated groups that had been treated for 14 days or longer, little evidence of tuberculosis was observed grossly. As a matter of fact, in 54 per cent of 72 animals that had been treated for 2 weeks or longer, no gross or microscopic lesions of tuberculosis were found The disease in the remaining 46 per cent of the treated animals was for the most part of minimal proportions

A summary of the average amount of tuberculosis in each of the 10 groups of treated and untreated animals expressed graphically on the basis of data obtained from a microscopic examination of the tissues of predilection is given in Figure 5

The information obtained from this experiment indicates that streptomycin was equally successful in suppressing the infection produced by the 7 recently isolated strains of human tubercle bacilli,

the 2 strains of bovine tubercle bacilli, and the laboratory stock strain H37Rv.

Although the number of different strains tested in these studies was small, the evidence indicative of the favorable effect of streptomycin against all of the strains is clear and unmistakable.

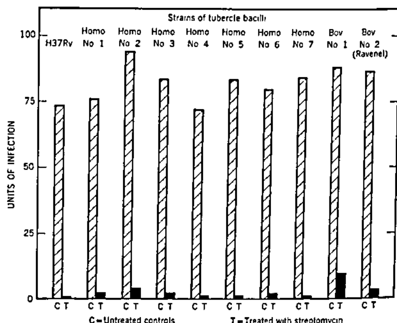


Fig 5 Comparative amounts of residual tuberculosis observed microscopically and expressed graphically in 10 groups of treated and untreated guinea pigs In each group, treatment with streptomycin was started on the fourteenth day after infection had been established and was continued for 54 days (9)

Frequency of Streptomycin Administration. In an experiment of some practical importance, an attempt was made to determine how frequently streptomycin should be administered to tuberculous guinea pigs in order to obtain a satisfactory therapeutic result Prior to this experiment, the medication schedule followed consisted in giving 6 mg of streptomycin daily per animal, divided into 4 equal doses injected at 6 hour intervals To determine whether the therapeutic effectiveness of streptomycin would be significantly influenced

by giving the drug less frequently, the following study was done (11,14).

Each of 64 guinea pigs was inoculated subcutaneously with 0 1 mg of human type of tubercle bacilli, strain H37Rv; 23 days later, and before any of the animals were treated, 10 of the infected animals were killed to determine the extent of the tuberculosis present It was found that the disease was recognizable on gross examination of the tissues of all of these animals The infection had disseminated in most instances to the spleen, liver, and lungs, and appeared definitely progressive These findings suggested the presence of well-established tuberculosis in all of the animals in the experiment

On the same day that the aforementioned animals were killed, 40 of the remaining 54 infected guinea pigs were divided into 4 groups of 10 animals each These 4 groups were treated with streptomycin The 14 remaining animals were the untreated controls.

The respective animals in each of the 4 groups of treated animals received streptomycin as follows group 1 received 2 mg 4 times daily; group 2, 4 mg twice daily, group 3, 8 mg once daily, and group 4, 4 mg 4 times daily every alternate week. The total dosage for each animal in all of the treated groups was the same The duration of the experiment was 83 days The period of treatment was 60 days

The results of this experiment showed that severe and widely disseminated destructive tuberculosis developed in the control group This apparently was responsible for the death of 50 per cent of the untreated animals during the period of observation. In the treated groups, the therapeutic effect of streptomycin was unmistakable. The efficacy of the drug was essentially equal in each of the 4 groups. Consequently, there was no evidence that in tuberculous guinea pigs streptomycin, to be effective, must be administered every 6 hours The fact that streptomycin was effective even when given every other week may be of some clinical significance.

From the results of this experiment it was concluded that in the treatment of tuberculous guinea pigs with streptomycin frequent administration of the drug is not essential Since this information became available, we have administered the drug once daily. The results have been satisfactory The clinical significance of these observations is important

Treatment Following Intravenous Inoculation The most impressive evidence that we have observed regarding the high potency of streptomycin *in vivo* against the human type of tubercle bacilli was obtained from the results of an experiment in which the animals were inoculated intravenously.

In this experiment, guinea pigs were infected with 1.0 mg., moist weight, of tubercle bacilli introduced directly into the circulation That the infection was exceedingly virulent was evident from the fact that the first of the 12 untreated controls died 11 days after infection and the last died on the twenty-seventh day. In contrast to the relatively rapid death of the animals that were not treated were the survival times of those that received streptomycin. Most of these lived many months after being infected, the last animal dying 341 days after inoculation (Fig. 6).

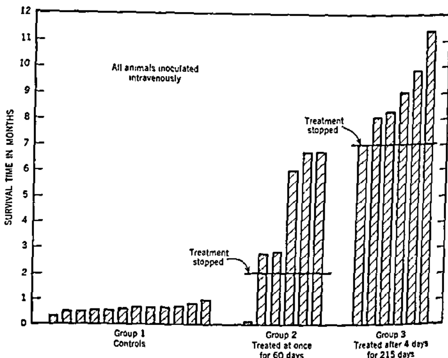


Fig 6 Comparative survival times of three groups of guinea pigs inoculated intravenously with tubercle bacilli Note the extended longevity of the animals in groups 2 and 3 which had received streptomycin (19)

This experiment, which is reported in detail elsewhere (see 19), has demonstrated dramatically the potency of the suppressive action of streptomycin on tubercle bacilli of the human type under extremely formidable conditions. Not only was the infective inoculum excessively large, but in addition the bacteria were introduced by the method most likely to produce a rapidly fulminating disease. In

spite of these unusually severe conditions, streptomycin greatly modified the expected course of the infection. Furthermore, this experiment demonstrated the ability of the drug to alter favorably and significantly the pathogenesis of a severe tuberculous infection in a host having little natural resistance to this disease. The results should dispel any doubts concerning the *in vivo* vulnerability of the tubercle bacillus to attack by chemical agents

Youmans and McCarter (60) found streptomycin to be effective in suppressing tuberculosis in mice previously inoculated intraveneously with 0 1 mg of human type of tubercle bacilli

Finally, it would appear that any drug capable of exerting even a limited deterrent action on a tuberculous infection of such lethal proportions as that following intravenous inoculation has impressive therapeutic possibilities Even though the shortcomings of streptomycin are fully recognized, the fact remains that the substance is capable of modifying favorably, and often dramatically, the pathogenesis of tuberculosis under extremely unfavorable circumstances

Significance of Experimental Results

The results obtained from our many studies to determine the antagonistic effects of streptomycin in experimental tuberculosis have demonstrated that this drug has a high potency against infections produced by tubercle bacilli In all the experiments, a highly susceptible species, the guinea pig, was used In addition, the conditions for successful treatment were made unusually difficult by delaying the beginning of treatment until the disease had progressed from 2 to 7 weeks This means that when treatment with streptomycin was started, the infection had attained considerable momentum and had become disseminated from the site of inoculation to the regional lymph nodes, spleen, liver, and lungs. Under natural conditions, a tuberculous disease of this character in guinea pigs is irreversible With streptomycin it has been possible in numerous experiments so to interfere with the natural progression of the disease that lesions present when treatment was begun resolved, healed by fibrosis, or calcified

Apropos of the regressive changes that occur in lesions of tuberculous animals treated with streptomycin, the character of the histopathologic alterations is significant. After treatment has been con-

tinued for a few weeks to several months, morphologic evidence characteristic of healing can be noted The lesions are no longer progressive, but frequently assume the appearance of a "hard tubercle" (inactive accumulations of epithelioid cells without central necrosis). Transformation of epithelioid cells to fibrocytes is seen, and lesions may even become hyalinized. These changes indicate the inability of the infection to advance. It would appear that the stimulating factor (the tubercle bacillus) responsible for the progressive state of the lesions is definitely subdued and that the natural processes of repair are operative Any substance that can accomplish changes of this character in the experimental animals and that is reasonably free from toxicity should be considered for clinical trial

However, it must be emphasized that, even though striking effects are exerted on the anatomic features of tuberculosis, the infective bacteria in the majority of animals are suppressed rather than killed by streptomycin It is probably true that streptomycin *in vivo* does render many tubercle bacilli nonviable; perhaps in some instances most of the organisms are killed Yet, though treatment may be continued for prolonged periods, a few tubercle bacilli usually persist This is true despite the absence of tissue changes indicative of lesions. This suggests that in an organ such as the spleen a few tubercle bacilli are taken up by monocytes, where, because of their intracellular position, they may be protected against the antibacterial action of the therapeutic agent However, the concentrations of streptomycin which are attained in the blood and tissues are not sufficiently high to be bactericidal. This persistence of the infective bacteria in animals in which tuberculosis can no longer be demonstrated cannot be explained on the basis of drug resistance. As a matter of fact, most cultures of tubercle bacilli obtained from spleens of successfully treated guinea pigs have not shown any increase in streptomycin resistance Perhaps if guinea pigs were able to exert a greater natural resistance to the tubercle bacillus the few bacteria that remain after streptomycin therapy would eventually be rendered harmless by natural processes.

Regardless of the shortcomings of streptomycin in comparison with the theoretically ideal tuberculochemotherapeutic agent, the data available leave no doubt that this agent is capable of a powerful deterrent action against extensive tuberculous infections The fact

that it has a low toxic potential and a high therapeutic index justifies a thorough exploration of its value in clinical tuberculosis.

Streptomycin in Clinical Tuberculosis*

Within a few months after the demonstration that streptomycin is capable of arresting tuberculosis in experimentally infected guinea pigs, this drug was first tested clinically on patients who had potentially fatal types of tuberculosis (29) To avoid undue toxic reactions with the crude preparations of streptomycin then available, it was first employed in extremely small doses, which were gradually increased. Though no early treated patients with tuberculous meningitis survived, it is possible to review their records and note that a recognizable clinical improvement was observed in a majority, despite the fact that the drug was of low potency and the dosages employed were extremely small, by more recent standards. Among the patients treated in the earlier phase of our studies a few were included who had severe, rapidly extending pulmonary tuberculosis that had developed after surgical procedures, such as thoracoplasty and pulmonary resection for tuberculosis In these patients, also, a clinical response was observed which in retrospect appears unmistakable. However, at that time, it seemed equally probable that the improvements which were noted might be spontaneous, and not due to specific drug therapy.

Within a year after streptomycin was announced in 1944, studies on its use in several types of tuberculosis of man were well under way at the Mayo Clinic, Mineral Springs Sanatorium (Cannon Falls, Minn ; Dr. Karl H Pfuetze, Medical Director), and the Rochester State Hospital This project was carried out in collaboration with Drs. H A. Burns and Magnus Peterson As mentioned previously, rather extensive studies on tuberculosis of guinea pigs had been completed in our laboratories Difficulties encountered by manu-

*The streptomycin used in our clinical trials was supplied through the generosity of Merck & Co , Inc , Rahway, N J ; Abbott Laboratories, North [illegible]

search Council, supplies received from September, 1946, to July, 1947, were allocated through the Committee on Therapy of the American Trudeau Society (Medical Section of the National Tuberculosis Association)

facturers in the production of streptomycin, and the obstacles to research imposed by the war then in progress, prevented many other investigators from entering the field for over another year. During 1946, interest in this field developed at a rapidly accelerating pace, and by the end of 1946 more than a score of institutions were engaged in clinical and experimental studies of streptomycin in tuberculosis

Clinical studies which have been completed (29–31), and others which are now in progress involving several institutions, have been co-ordinated to an unprecedented degree through the auspices of a series of voluntary and official agencies on a nationwide basis

The agencies include the Medical Section of the National Tuberculosis Association (American Trudeau Society), the United States Veterans Administration, the United States Army, the United States Navy, the Research Grants Division of the National Institute of Health, and the Tuberculosis Control Division of the United States Public Health Service Each of these agencies has made available to other groups complete information as to the studies carried out. The advice of representatives from all agencies has been utilized in the planning and execution of a series of therapeutic trials which we believe to be unprecedented in the history of medical research. This arrangement has made possible the confirmation of many important facts before publication and has resolved any uncertainties which might have seriously confused the medical literature, had not the basis for all important differences been revealed by personal consultation between the many research workers involved. We wish to express our appreciation for aid and advice received from many research workers engaged in this field, and especially to the following Drs John Barnwell, Arthur Walker, Paul Bunn, Walsh McDermott, H McLeod Riggins, and their colleagues

Although we and our co-workers at the Mayo Clinic and the Mayo Foundation, together with our close collaborator, Dr Karl H. Pfuetze, of the Mineral Springs Sanatorium, treated between 1945 and 1947 approximately 150 patients who had tuberculosis, we had the good fortune of being able adequately to review several hundred additional cases, in most of which the patients were treated under the auspices of the tuberculosis service of the Veterans Administration and the American Trudeau Society

Miliary Tuberculosis. Acute hematogenous miliary tuberculosis in man more closely resembles experimental tuberculosis of guinea pigs than does any other form of human infection with Mycobacterium tuberculosis As is well known, the natural course of the disease is almost invariably fatal, and we had been unable to modify

the course of the disease significantly by any method of treatment which we had attempted prior to the development of streptomycin.

Milgram, Levitt and Unna (42) have described the arrest of miliary tuberculosis in children by using 4,2'-diaminophenyl-5'-thiazolesulfone, known commercially as promizole, which was found in our laboratories to be effective in treatment of experimental tuberculosis (16) and which we have tried clinically, with indifferent results, in other forms of tuberculosis.

The objective manifestations of the disease can be followed readily by roentgenograms of the thorax, which indicate clearly the characteristic pulmonary lesions Furthermore, it is occasionally possible to observe tubercles in the retina directly by means of the ophthalmoscope.

When streptomycin treatment is employed in a case of acute progressive hematogenous miliary tuberculosis, clinical evidences of response to treatment may often be observed within the first few days There is a slow but consistent tendency for fever to decline and frequently the "typhoid state" improves correspondingly. Roentgenographic changes in pulmonary lesions are not usually demonstrable until after 3 or 4 weeks of treatment, when the lesions begin to appear more discrete and circumscribed, the previously hazy borders of individual masses of tubercles becoming sharply demarcated and possibly of a slightly increased density Within the next few weeks, there is a striking tendency to resolution of these lesions, as observed roentgenographically Rather frequently, these miliary pulmonary lesions are scarcely detectable in roentgenograms after 2 months of treatment, and in some instances a complete remission is observed, roentgenographically, clinically, and bacteriologically The patient's sense of well being may return to normal, tubercle bacilli which may have been previously demonstrated in gastric contents or sputum have disappeared, and without knowledge of the patient's previous illness, a diagnosis of miliary tuberculosis could not be suspected

We have observed that in patients with miliary tuberculosis, whose disease has been brought to a state of arrest by streptomycin, the disseminated lesions of miliary calcification, such as are occasionally noticed in roentgenograms of the thorax of persons who are well, have not developed Such foci of miliary calcifications have occasionally been attributed by some observers to healed miliary tuberculosis but evidence secured from our studies of subjects who had been treated with streptomycin casts doubt on this explanation of the

facturers in the production of streptomycin, and the obstacles to research imposed by the war then in progress, prevented many other investigators from entering the field for over another year. During 1946, interest in this field developed at a rapidly accelerating pace, and by the end of 1946 more than a score of institutions were engaged in clinical and experimental studies of streptomycin in tuberculosis.

Clinical studies which have been completed (29–31), and others which are now in progress involving several institutions, have been co-ordinated to an unprecedented degree through the auspices of a series of voluntary and official agencies on a nationwide basis

The agencies include the Medical Section of the National Tuberculosis Association (American Trudeau Society), the United States Veterans Administration, the United States Army, the United States Navy, the Research Grants Division of the National Institute of Health, and the Tuberculosis Control Division of the United States Public Health Service Each of these agencies has made available to other groups complete information as to the studies carried out The advice of representatives from all agencies has been utilized in the planning and execution of a series of therapeutic trials which we believe to be unprecedented in the history of medical research This arrangement has made possible the confirmation of many important facts before publication and has resolved any uncertainties which might have seriously confused the medical literature, had not the basis for all important differences been revealed by personal consultation between the many research workers involved We wish to express our appreciation for aid and advice received from many research workers engaged in this field, and especially to the following Drs John Barnwell, Arthur Walker, Paul Bunn, Walsh McDermott, H McLeod Riggins, and their colleagues

Although we and our co-workers at the Mayo Clinic and the Mayo Foundation, together with our close collaborator, Dr. Karl H Pfuetze, of the Mineral Springs Sanatorium, treated between 1945 and 1947 approximately 150 patients who had tuberculosis, we had the good fortune of being able adequately to review several hundred additional cases, in most of which the patients were treated under the auspices of the tuberculosis service of the Veterans Administration and the American Trudeau Society

Miliary Tuberculosis. Acute hematogenous miliary tuberculosis in man more closely resembles experimental tuberculosis of guinea pigs than does any other form of human infection with Mycobacterium tuberculosis. As is well known, the natural course of the disease is almost invariably fatal, and we had been unable to modify

even while healing is in progress in the lungs and elsewhere In other instances, acute tuberculous meningitis has developed in patients who were receiving streptomycin for miliary tuberculosis, and who had previously been making satisfactory progress Such tuberculous meningitis may or may not respond to intrathecal treatment with streptomycin while the parenteral treatment is being continued. In a few instances, miliary tuberculosis, which was temporarily under control, has recurred after cessation of treatment, or even while treatment was under way, and has progressed despite further treatment, apparently as a result of the presence of drug-resistant tubercle bacilli in the lesions.

Because of the highly fatal nature of miliary tuberculosis, it is recommended that maximal tolerated doses of streptomycin be employed in the treatment of this disease, efforts being made to avoid only the more serious and the more permanent types of drug toxicity. We believe that the usual dose for an adult person of average size with miliary tuberculosis should be approximately 2 Gm. per day. It is recommended that in the treatment of this disease the suggested dose should be divided into 2 portions, which are injected at intervals of 12 hours. It has not yet been determined whether smaller doses would be equally effective in the treatment of miliary tuberculosis.

Present studies have not been sufficiently long-continued to enable one to know what percentage of individuals may survive acute miliary tubercuolsis as a result of streptomycin treatment. It is suggested, therefore, that a guarded prognosis be given regarding such patients However, since there is no other treatment known which is capable of bringing about a comparable remission of this disease, we believe that streptomycin therapy is mandatory in all cases of proved miliary tuberculosis.

Mention should be made of the fact that nontuberculous pulmonary infiltrations may simulate miliary tuberculosis The recognition of these confusing lesions calls for the exercise of wise clinical judgment.

Tuberculous Meningitis.* Tuberculous meningitis resembles

* Since the preparation of this manuscript, several important publications on tuberculous meningitis have appeared, among which the following should be consulted Debré *et al* (5a); McDermott *et al* (40a); and a report of the Medical Research Council (40b)

presence of calcification of miliary foci It is probable that these lesions of miliary calcification are the result of some other disease than tuberculosis including perhaps a benign form of histoplasmosis

Unfortunately, the remissions which have so frequently followed treatment of miliary tuberculosis with streptomycin are not always sustained. It is believed, however, that a considerable percentage of

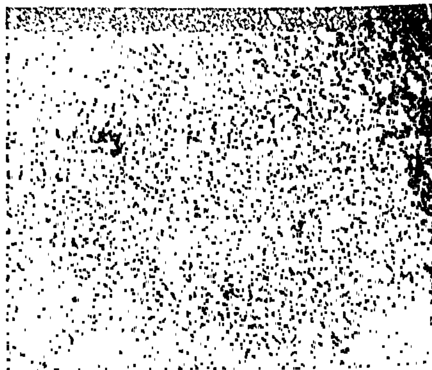


Fig 7 Region of active tuberculosis in cerebrum of a man who had died after treatment with streptomycin for miliary tuberculosis Miliary lesions (×64) in the lungs, liver, and spleen showed morphologic evidence of healing (2)

patients who have very early miliary tuberculosis, may possibly have their disease actually cured by streptomycin treatment In other instances, the disease has progressed, especially in the central nervous system, producing what might be termed a tuberculous encephalitis (2) (Fig 7) It has been shown (2) that streptomycin does not penetrate the substance of the brain, it is probably for this reason that the disease tends to advance in the central nervous system

disease, and suggest that pathologists make special effort to determine if any anatomic explanation can be found for this phenomenon.

Improvement in the cerebrospinal fluid findings of patients who are responding clinically to streptomycin treatment is much slower than is clinical response, and indeed the actual irritation produced by intrathecal injection of streptomycin may appear to increase the number of cells noted on microscopic examination of the cerebrospinal fluid. The sugar content of the fluid tends to increase toward the normal level, and during succeeding weeks the cell count gradually declines. The elevated protein content of the fluid, however, may remain abnormal for many months We have observed only a few instances in which during treatment the cerebrospinal fluid became normal in every respect: microscopically, chemically, and bacteriologically However, the improvement in the cerebrospinal fluid may continue after cessation of treatment, and the fluid may become normal several weeks later.

In view of the frequent responses to treatment which may be observed in this disease and in view of the probability that some few patients at least may actually be cured of tuberculous meningitis by streptomycin treatment, we believe that in all cases of tuberculous meningitis, proved or suspected, intensive treatment with streptomycin should be instituted.

The dose of streptomycin recommended for treatment of tuberculous meningitis, to be administered by the intramuscular route, is similar to that mentioned in the preceding section on miliary tuberculosis The average dose for adults will probably be about 2 Gm per day, divided into two doses. The duration of therapy should probably be at least 3 months, and possibly in some instances as long as 4 to 6 months Children have usually received somewhat larger doses than would be computed on a body weight basis, when compared with adults The minimal intramuscular dose which we have employed for infants has usually been 0 8 to 1 Gm. per day, and the intrathecal dose 25 to 50 mg per day.

The intrathecal administration of streptomycin is advised in cases of tuberculous meningitis, as we have not observed any patient whose disease was arrested for prolonged periods by intramuscular streptomycin administration alone The intrathecal dose used for adults has varied from 25 to 100 mg, injected either every day or every other day, and continued for several weeks Severe reactions have

miliary tuberculosis in being a disease which has hitherto been completely refractory to all therapeutic efforts. Only a very few patients who had this disease have been known to recover spontaneously, and with regard to some of these the diagnosis was in doubt. This disease may or may not be associated with demonstrable generalized hematogenous miliary tuberculosis. Preliminary studies suggest that in those cases in which tuberculous meningitis is not associated with demonstrable miliary tuberculosis the meningitis is more responsive to streptomycin treatment than in those which are associated with miliary tuberculosis, as shown by roentgenograms of the thorax. Those patients whose symptoms of meningitis have been of only a few days' duration are probably more likely to respond to streptomycin treatment than are patients in whom the disease has existed for a longer period. Because of the importance of early treatment in this form of tuberculous infections, it will frequently be necessary to treat patients with meningitis on the basis of a presumptive diagnosis of tuberculous etiology even before bacteriologic proof can be obtained by inoculation of guinea pigs or by cultures of tubercle bacilli obtained from cerebrospinal fluid.

For a consideration of the clinical and pathologic aspects of tuberculous meningitis, the recent paper by Smith and Daniel (51) may be consulted. These writers stress the importance of early diagnosis if successful chemotherapy of tuberculosis of the central nervous system is to be realized.

The clinical response of tuberculous meningitis to streptomycin therapy is sometimes most dramatic after the first week or two of treatment. When the drug is given both parenterally and intraspinally, the patient, who may have been extremely ill, in coma, with high fever and all other clinical and neurologic symptoms of this imminently fatal infection, may improve slowly at first and later more rapidly to a point of essential normality. The sensorium clears, the patient becomes alert and free of discomfort, and regains his appetite and other evidences of well-being. The temperature curve declines rather slowly for the first few weeks, and may even remain moderately elevated for several weeks.

A considerable percentage of patients who have tuberculous meningitis fail to make any recognizable response to treatment with streptomycin and die during the first week or two of treatment. We suspect that these cases represent a distinctly different type of the

more than 90 per cent of all deaths from infection with Mycobacterium tuberculosis in the United States. Because of this fact, the potentialities of streptomycin treatment are of the utmost significance in this form of the disease. Unfortunately, pulmonary tuberculosis varies widely in its clinical manifestations and the course of the disease often cannot be predicted accurately. Therefore, when modification of the course of the disease is observed, there is frequently the possibility that this altered course may have been spontaneous rather than due to any form of specific treatment. Great care must be exercised in interpreting the significance of clinical improvement of pulmonary tuberculosis under any form of treatment, since tuberculosis of the lungs tends to improve spontaneously in a high percentage of cases. For this reason, we have chosen in our studies of pulmonary tuberculosis to treat those patients whose disease had been progressive despite conservative treatment, including rest in bed. Although the disease usually was quite extensive, we chose for treatment, whenever possible, those patients in whom we believed that the changes wrought were likely to be reversible If our opinion that streptomycin, like other antibacterial agents, is largely suppressive in action should prove to be correct, the limitations to its usefulness in many forms of pulmonary tuberculosis will be considerable

Unquestionably, many more studies will be required before it will be possible to choose wisely which patients with pulmonary tuberculosis are most likely to respond to streptomycin treatment. We believe that some types of pulmonary tuberculosis will require study by accurately controlled methods of investigation to determine more definitely what the indication for streptomycin treatment may be

In our present state of incomplete knowledge, we believe that streptomycin treatment should be reserved for those patients whose disease appears to be largely of exudative type. Before streptomycin treatment is instituted, the physician should have decided that other forms of treatment available are not likely to achieve arrest of the disease, and that in his opinion the disease would probably be progressive under conventional treatment. The indications for streptomycin treatment of pulmonary tuberculosis are being broadened now that streptomycin is widely available, its cost has been reduced, and the toxic potentialities of the drug are better understood

The symptomatic response of patients with extensive pulmonary

frequently been encountered after intrathecal administration of streptomycin, some lots of streptomycin being much more irritating than others. It is recommended that for intrathecal injection highly purified streptomycin be utilized, when this is available We have administered streptomycin by cisternal puncture as well as by lumbar puncture, but have not determined which route is preferable from the standpoint of efficacy.

Unfortunately, in a rather large percentage of patients who have tuberculous meningitis the degree of remission described in preceding paragraphs may not be obtained In some instances, there is no evidence of improvement whatever, and the patient may die within a week or two after institution of treatment. Others linger on for several weeks or several months, and eventually die without having achieved complete remission. However, in an appreciable percentage of cases in which tuberculous meningitis has been diagnosed early, there will be complete clinical remission even though abnormalities may persist in the cerebrospinal fluid for prolonged periods of time.

The eventual prognosis in cases of tuberculous meningitis remains exceedingly grave, for late recurrences, or what might possibly be regarded as second attacks of tuberculous meningitis, have been noted by us even a year after apparently complete remission Even those patients whose cerebrospinal fluid has become normal may suffer a relapse after a remission of several months' duration. However, actual cure of tuberculous meningitis is likely to be achieved in a small percentage of cases.

Patients who have tuberculous meningitis are likely to suffer from partial or complete loss of hearing In some instances, this may be due to inflammatory reaction about the auditory nerve or damage to its nucleus resulting from tuberculosis In most instances, the loss of hearing must be regarded as a manifestation of streptomycin toxicity, probably due to the fact that large dosages were used for prolonged periods It is also possible that intrathecal injection of the drug may increase the neurotoxicity of streptomycin Even when deafness appears, we have been reluctant to discontinue treatment because of the highly fatal nature of tuberculous meningitis. Further experience will be necessary to know whether the incidence of deafness can be reduced or abolished by reducing dosage or by intermittent treatment.

Pulmonary Tuberculosis. Pulmonary tuberculosis accounts for

apparent. It is widely believed that streptomycin may widen the indications for more radical surgical procedures in cases of pulmonary tuberculosis, may shorten the period of care in a sanatorium, and may increase the effectiveness of more conservative types of treatment.

Tracheobronchial Tuberculosis. Progressive ulcerating and hyperplastic lesions of the tracheobronchial tree have been recognized in recent years as constituting a grave complication of pulmonary tuberculosis and one which frequently fails to respond to all efforts at treatment. Response of such lesions to streptomycin therapy can be observed directly by the bronchoscopist, and hence they constitute ideal lesions for study of antibacterial remedies against tuberculosis.

In our experience, ulcerating and hyperplastic tuberculosis of the trachea and larger bronchi has usually been treated with intramuscularly administered streptomycin which may or may not be combined with streptomycin aerosol. The healing of this kind of lesions is usually very prompt and consistent after streptomycin treatment, often being quite evident within two weeks and complete within four to six weeks Furthermore, recurrence of such lesions after treatment has been extremely rare in our experience thus far, and this group of cases has constituted one of the most satisfactory types for treatment with streptomycin It is important to distinguish sharply between active ulcerating lesions and fibrous bronchial strictures which may or may not produce atelectasis. The latter lesions are the results of previous infection, and the mechanical obstruction obviously cannot be resolved by antibacterial therapy. Indeed, we have observed that healing of ulcerating lesions as a result of streptomycin therapy frequently will produce fibrous bronchial strictures which may require repeated bronchoscopic dilatation, and at times pulmonary resection when this can be achieved

Laryngeal Tuberculosis. Tuberculosis of the larynx has many pathologic and clinical characteristics which identify it with hyperplastic and ulcerating lesions of the tracheobronchial tree. The response to streptomycin therapy has been highly gratifying in most instances which we and our colleagues have studied thus far (20). The symptoms of laryngeal tuberculosis are so severe in many instances that we believe streptomycin therapy should be given when available, even when it appears that permanent arrest of the asso-

tuberculosis to streptomycin therapy is sometimes striking The improvement or disappearance of cough and expectoration has been repeatedly observed, fever may tend to decline slowly, and the patient's appetite may steadily improve long before any change in the roentgenogram is noted These clinical improvements are undoubtedly due in part to the improved morale which is incident to the institution of any new form of treatment in the case of a discouraged patient. This particularly is true if he has been impressed with the fact that he is being given a rare and expensive drug of reputed merit. However, most physicians who have had experience with streptomycin in the treatment of tuberculosis are convinced that the improvement of objective symptoms is due in large part to the antibacterial effect of streptomycin on the multiplication of tubercle bacilli in the lesions of pulmonary tuberculosis.

The improvements noted in pulmonary lesions of tuberculosis on roentgenographic examination appear to be exactly what would be anticipated on the basis of the usual concepts of the pathologic changes which produce the shadows in the roentgenogram Those types of pulmonary infiltration which are diffuse and widely disseminated probably are in close contact with the circulation, and these are the types of shadows which ordinarily first show improvement roentgenographically after streptomycin treatment. Thin-walled cavities of recent origin may tend to diminish in size and even disappear after several weeks of streptomycin treatment. Thick-walled cavities of extended duration, and regions of coarse fibrosis, usually show little or no change after streptomycin treatment. Occasionally, the physician is surprised to note, however, that even old lesions may slowly improve, and this improvement may continue during the months following the course of streptomycin treatment. It is probable that in such instances the improvement noted is not alone a direct effect of streptomycin but is due to a mobilization of the patient's defensive forces which had previously been inoperative because of an overwhelming population of tubercle bacilli.

During the early research phases of the problem, streptomycin was usually not combined with collapse therapy, in order to obviate the difficulty of determining the relative value of two therapeutic procedures utilized simultaneously However, the practical possibilities of combining streptomycin with collapse therapy and surgical excision of tuberculous pulmonary lesions are immediately

ute to a more rapid healing of these lesions Penicillin therapy alone has sometimes been successful in treatment of tuberculous sinuses.

Miscellaneous Lesions of Tuberculosis. Tuberculous enteritis associated with extensive pulmonary tuberculosis has in a striking degree appeared to respond to streptomycin treatment In a few cases, tuberculous pleuritis with effusion has likewise improved promptly; but, since this lesion tends to improve spontaneously, accurate judgment concerning the role of streptomycin in the results is not possible at present. Tuberculous peritonitis has also appeared to respond to treatment with streptomycin.

Tuberculosis of bones and joints offers a wide variety of pathologic conditions second in complexity only to pulmonary tuberculosis, and observations in this field are incomplete at present. However, results appear to be entirely consistent with what might be anticipated on the basis of pathologic changes present. Tuberculosis of the synovial membrane and tuberculosis associated with draining sinuses have appeared to respond most favorably. Large paravertebral abscesses have not improved. As in the case of pulmonary tuberculosis, it appears that tuberculosis of the skeletal system will frequently require surgical, as well as antibacterial, treatment.

In the past, tuberculosis of the genitourinary tract has been one of the most difficult types of tuberculosis to treat because of the poor defensive mechanisms which the human body has against tuberculosis in these organs Spontaneous healing of renal tuberculosis rarely occurs, and unless the disease is in tissues which can be excised surgically, the ultimate prognosis is very poor If our present hypothesis that streptomycin serves as a suppressive agent and only for a limited period is correct, it might be anticipated that such treatment would be of symptomatic value only in treating advanced tuberculosis of the genitourinary tract, especially of kidneys and bladder We have administered this drug chiefly to patients who had recurrent tuberculosis in a solitary kidney after nephrectomy at some previous date (5) Possibly we have chosen for treatment patients whose disease was in a too far advanced stage for any permanent favorable results to be expected Far more extensive studies will be required before the place of streptomycin in the treatment of tuberculosis of the genitourinary tract can be definitely determined. At present, it appears that its effects are largely palliative rather than curative in advanced types of the disease. Studies are now under

ciated pulmonary tuberculosis may be impossible. The symptomatic improvement is frequently very impressive and rapidly achieved. Patients who have severe dysphagia and laryngeal pain may experience relief of symptoms within a few days after institution of streptomycin treatment. This treatment should consist in intramuscular administration of streptomycin which may be combined with either streptomycin aerosol or streptomycin applied topically by means of an atomizer, utilizing solutions similar to those recommended for aerosol administration.

Tuberculous Empyema. We and our associates have had disappointing results in treating tuberculous empyema with streptomycin. Experiments are now being conducted on the use of streptomycin in combination with other antibacterial agents, in the hope that better results may be achieved. In previous communications we have suggested the possibility that the failure of streptomycin in cases of tuberculous empyema may be due to the hydrogen ion concentration in the pleural cavity, but subsequent experience has made it appear that this conclusion was probably incorrect.

Scrofuloderma and Tuberculous Draining Sinuses. Streptomycin administered parenterally has proved to be remarkably consistent in promptly closing cutaneous sinuses of tuberculous origin, almost regardless of the nature of the underlying lesion except that streptomycin is usually ineffective if the lesion is one of tuberculous empyema In the majority of cases, the draining sinuses treated by us (18) have been due to underlying tuberculous lymphadenitis, or tuberculosis of the thoracic wall, or, in a few instances, to tuberculous lesions of bones and joints These sinuses had been present for periods varying from several months to several years; only those cases were treated in which it appeared that there was no tendency to spontaneous healing Discharge usually ceases within 1 to 3 weeks, and a scab occludes the sinus for several more weeks When this scab becomes dislodged, the sinus is found to be closed We have usually continued treatment for 2 to 4 weeks after superficial healing has been observed. Recurrences are infrequent when this procedure is followed It is our belief at the present time that the existence of tuberculous draining sinuses is a definite indication for institution of streptomycin treatment In a few instances in which closure was less rapid than anticipated and bacteriologic studies revealed the presence of mixed infection, penicillin therapy has appeared to contrib-

eventually result in the arrest of the disease The fact that tissue changes of this character could be recognized in many of the animals treated with streptomycin provides additional evidence that an effective drug treatment for tuberculosis does more than prevent further progress of the infection, it also suppresses the bacterial activity sufficiently to permit the natural processes of healing and repair to become operative.



Fig 8 Granulomatous nodule in lung of man who had been treated for six weeks with streptomycin for miliary tuberculosis The lesion (×120) no longer resembles tuberculosis, it is nonspecific in character and healing tendencies predominate (9)

A study (2) of tissues from a few patients who had died in the earlier phase of our investigations makes it evident that morphologic changes similar to those described in tuberculous animals treated with streptomycin may also occur in tuberculous human beings treated with this drug The material was from cases of either miliary

way on combining streptomycin with other antibacterial agents, and preliminary results suggest the possibility that better clinical results will be achieved by such combination.

Tuberculosis of the skin is likewise a very complicated problem, since cutaneous lesions ascribed to tuberculosis cannot always be proved to be of such origin; tubercle bacilli may not be present and the manifestations may be ascribed to hypersensitivity reaction rather than to actual infection. In other instances, such as lupus vulgaris, tubercle bacilli are of very low virulence and atypical in character *It is recommended that more extensive studies of cutaneous tuberculosis* be undertaken with the closest possible cooperation with clinical pathologists.

Morphologic Evidence of Therapeutic Effect

The extreme difficulty of appraising the therapeutic effect of any drug used in the treatment of clinical tuberculosis is recognized by all who understand the natural history of this disease Lesions that are exteriorized or that can be examined visually by proper instrumentation can be inspected frequently and changes can be recorded. But when the disease affects tissue within body cavities the state of the disease can only be determined by roentgenographic examination and by clinical signs. In the experimental animal, the investigator has the distinct advantage of being able to examine all tissues not only grossly but microscopically and thus to obtain important additional evidence that is frequently not available from clinical investigations

Since our many investigations of streptomycin in experimental tuberculosis *have provided a wealth of material for histopathologic* studies, we have been able—as mentioned previously—to demonstrate that tuberculous lesions in guinea pigs treated with streptomycin are subject to striking and significant morphologic alterations These changes, which are readily demonstrable microscopically, are those that characterize regression or healing (Figs 3 and 4). After treatment with streptomycin, the morbid process—if present at all—is no longer that of a destructive advancing disease but instead is a process that has become quiescent, is regressing, or has the appearance of a nonspecific granulomatous reaction We believe these changes to be consistent with the repair and healing that

eventually result in the arrest of the disease. The fact that tissue changes of this character could be recognized in many of the animals treated with streptomycin provides additional evidence that an effective drug treatment for tuberculosis does more than prevent further progress of the infection; it also suppresses the bacterial activity sufficiently to permit the natural processes of healing and repair to become operative.



Fig 8 Granulomatous nodule in lung of man who had been treated for six weeks with streptomycin for miliary tuberculosis The lesion (×120) no longer resembles tuberculosis, it is nonspecific in character and healing tendencies predominate (9).

A study (2) of tissues from a few patients who had died in the earlier phase of our investigations makes it evident that morphologic changes similar to those described in tuberculous animals treated with streptomycin may also occur in tuberculous human beings treated with this drug The material was from cases of either miliary

or meningeal tuberculosis, or a combination of the two forms of the disease. Although these patients died, treatment in most instances extended the duration of life considerably beyond that of similar patients who were not treated with streptomycin. Definite and con-

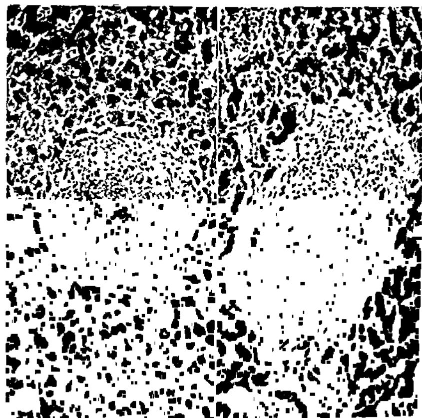


Fig. 9. Hepatic lesions of two human subjects who had had miliary tuberculosis. (*a*) progressive, necrotizing lesions (×110) from subject who had not received chemotherapy; (*b*) dense, hyalinized, nonactive nodule (×120) from subject who had been treated with streptomycin.

vincing morphologic signs of the effect of treatment were observed in the lungs, liver, and spleen. The changes which we interpreted as indicative of regression and healing were (*1*) contraction of the lesions, resulting in irregular fibrotic scars, many without the usual histologic features of tuberculous lesions (Fig. 8); (*2*) disappearance of

epithelioid elements; (3) reduction or absence of caseation, and (4) fibrosis and hyalinization (Fig. 9).

These findings, which have been confirmed by others (24), provide evidence to substantiate the clinical, roentgenographic, and laboratory data indicating that streptomycin does exert a favorable influence on the disease

Streptomycin Resistance

The phenomenon of drug resistance has been recognized since the days of Ehrlich, when it was observed that mice infected with trypanosomes and treated with parafuchsin required increasing amounts of the drug to banish the parasite from the blood after successive relapses (53) With the development and widespread use of the newer chemotherapeutic agents, examples of drug-resistant bacteria have been reported frequently (A résumé of microbial resistance to drugs used in chemotherapy will be found in the recent paper by Selbie (53)) The agents which may give rise to drug-resistant bacteria are varied, and include proflavine, gramicidin, tyrothricin, penicillin, streptomycin, and the sulfonamide compounds Clinical experience has shown that streptomycin therapy frequently and rapidly leads to the appearance of drug-fast strains of previously sensitive bacteria The problem should be of lesser importance in acute infections, which require chemotherapy for a relatively short period, except for the fact that the causative organisms of such acute diseases usually multiply at a very rapid rate The bacillus of tuberculosis multiplies slowly, and the development of a drug-fast population of Mycobacterium tuberculosis requires a much longer period than is the case with other organisms sensitive to streptomycin

The mechanisms underlying the development of drug resistance have not been entirely elucidated. Demerec (6) has advanced evidence to support the view that drug resistance is the result of the capacity of bacteria to undergo spontaneous variation or mutation. While one may assume that the vast majority of a population of tubercle bacilli is sensitive to a given drug, a few variants are present that are drug resistant even before treatment is started (48) When the bacteria come in contact with a chemotherapeutic substance, a

gradual elimination of the more sensitive cells occurs so that eventually only the more resistant cells are propagated.

Tubercle bacilli have become resistant to streptomycin by *in vitro* procedures (61) and in tuberculous guinea pigs treated with streptomycin (35). The first demonstration of this phenomenon in tuberculous human beings treated with this drug was reported by Youmans and associates (61). In our experience (19) the change from streptomycin sensitivity to streptomycin resistance of tubercle bacilli requires in guinea pigs a rather prolonged exposure to the drug (5–6 months). So far as we know, the streptomycin-resistant state of tubercle bacilli persists after repeated subculture and storage in the refrigerator. In addition, inoculation of guinea pigs with a streptomycin-resistant culture of tubercle bacilli and subsequent residence of the bacteria in the infected animals for 10 or more weeks did not reduce the degree of resistance of the bacteria to streptomycin (36) Furthermore, tuberculosis in guinea pigs and mice induced by streptomycin-resistant tubercle bacilli does not respond to streptomycin therapy (18,57)

In clinical studies with streptomycin, the question of "drug fastness" has appeared to be one of the most serious shortcomings of this type of therapy Some data have been obtained which were the subject of a preliminary report (61) *

Observations were made *in vitro* on 12 strains of tubercle bacilli isolated from a like number of patients before and after treatment with streptomycin. The tests of sensitivity disclosed that after treatment 8 of the 12 strains were markedly resistant to streptomycin In 7 of the 8 strains the resistance had increased by at least 500 to 1,000 times These observations have been confirmed by several other investigators

The studies just mentioned (61) demonstrate that in human

* Recently, our associate, Dr A G Karlson (34) has observed that in some cases streptomycin-sensitive cultures of tubercle bacilli may be obtained from materials from patients previously harboring streptomycin-resistant strains of tubercle bacilli Such patients had usually been treated for several months with streptomycin At the end of the period of treatment, the bacterial population was markedly resistant to the drug Karlson's observation that a previously streptomycin-resistant population of tubercle bacilli may eventually be replaced by one that is again streptomycin-sensitive is of great interest and has important clinical implications

beings after treatment with streptomycin tubercle bacilli, like various other bacteria, may become highly resistant to streptomycin. Unless methods are found to prevent or to delay the appearance of resistant strains, it appears that acquired resistance to streptomycin by tubercle bacilli becomes an obstacle to the complete realization of the therapeutic possibilities of this drug

We have conducted one study to determine if infections in guinea pigs produced by tubercle bacilli having a marked *in vitro* resistance to streptomycin would respond to streptomycin therapy (8,18).

In this study, tubercle bacilli with a normal sensitivity to streptomycin were obtained from a patient before treatment with streptomycin was started and were maintained in culture Subsequently, a culture of tubercle bacilli resistant to streptomycin *in vitro* was obtained from the same patient after treatment for 4 months with streptomycin Two experiments were done concurrently In one, guinea pigs were infected with the sensitive culture, in the other, similar guinea pigs were infected with the resistant culture Treatment with streptomycin of 10 animals in each experiment was begun 20 days after inoculation, and 10 additional infected animals were maintained as untreated controls Treatment was continued daily until all of the untreated controls had died (approximately 23 weeks) The results of this study showed that the disease in the animals infected with the streptomycin-sensitive culture responded favorably to treatment in the usual manner The disease in the animals infected with the streptomycin-resistant culture failed to yield to treatment In this instance, the amount and character of the tuberculosis in the untreated controls and in the treated group were comparable It was concluded that infections in guinea pigs induced by tubercle bacilli resistant *in vitro* to streptomycin are refractory to treatment with this antibiotic

Although the results of the experiments on animals showed definitely that highly resistant tubercle bacilli obtained from a patient were not amenable to the antagonistic action of streptomycin, information on the subsequent history of the patient from whom the bacilli were obtained is of more than ordinary interest After treatment with streptomycin was stopped, the patient continued to improve, numerous specimens obtained by gastric lavage failed to produce tuberculosis in guinea pigs, and at the present time, 30 months after treatment with streptomycin was discontinued, there has been no detectable reactivation of the disease

In considering the question of streptomycin resistance and its influence on the clinical use of the drug in tuberculosis, certain important factors should be recognized First, it appears to be true that

in clinical tuberculosis the great majority of strains of Mycobacterium tuberculosis have a relatively low resistance to streptomycin at the time when treatment is started (48). Consequently, in some types of cases the expected therapeutic benefits of the drug will be obtained before the streptomycin-resistant tubercle bacilli predominate. This may occur *after treatment for several weeks* or even several months

Secondly, and of most importance, during the period of treatment when tubercle bacilli of low resistance constitute most of the bacterial population, the suppression of the organisms—and perhaps the killing of some—enables the natural defenses of the patient to become, in most cases, effectively operative. As a consequence, there are set in motion the complex mechanisms of resistance and repair which were latent or suppressed as long as the large majority of the infective bacteria were undisturbed in their natural progression Once activated as an indirect result of the action of streptomycin *on the sensitive bacteria, the forces of repair seem, in most instances,* to continue effectively in operation. After streptomycin therapy is discontinued, even though highly streptomycin-resistant tubercle bacilli can, for a time at least, be isolated, the forces of resistance and repair are often expressed in the continued betterment of the clinical course of the disease

Tuberculous patients whose improvement fails to continue after treatment with streptomycin is stopped, are likely to be individuals who lack the intrinsic potential to mobilize the mechanism of natural defense against the disease effectively There has been a rather high percentage of cases of recurrent disease following temporary suppression of the disease by streptomycin treatment We believe it probable that this is due to the fact that most physicians have thus far made use of streptomycin more frequently for patients who were judged to have low resistance to the disease

In view of the incomplete information as to the exact significance of resistance of tubercle bacilli to streptomycin, final conclusions regarding the relationship of this phenomenon to the therapeutic potential of the drug clinically must be deferred However, that tubercle bacilli markedly resistant to streptomycin may occur in patients treated with the drug should be recognized by all who use streptomycin clinically Bacteriologic assays to determine sensitivity after treatment has begun are desirable if streptomycin therapy is to

be employed most intelligently, but the methods now utilized for such testing should be carefully examined to determine their adequacy.

The question of how best to overcome the obstacle of streptomycin resistance of tubercle bacilli is of the greatest importance to the future use of this drug. It has been suggested that the problem be met by giving exceedingly large doses of the drug so as to provide blood levels in excess of what would be necessary to suppress the most resistant strains *in vitro*. This has not proved to be a successful approach when doses of 3 Gm per day have been employed. It should also be pointed out that there is unlikely to be a close correlation between the levels of streptomycin in the blood and the amount of the drug that penetrates caseous lesions in which tubercle bacilli may be numerous

A more logical approach to the problem would seem to be to use, as the therapeutic regimen, a combination of two or more drugs, each having a high specificity for tubercle bacilli Streptomycin utilized simultaneously with another (as yet unknown) antibiotic agent might prove exceedingly effective Streptomycin combined with sulfone compounds and other synthetic antibacterial drugs offers possibilities which are now being explored in our institution* and elsewhere Smith and McClosky (55) have reported that the combined effect of promin and streptomycin in treating tuberculous guinea pigs was greater than was the effect of treatment by either substance alone The rationale of using a combination of dissimilar drugs to meet the obstacle of drug-resistant tubercle bacilli appears to be sound It has been observed frequently that strains of bacteria (other than tubercle bacilli) that are resistant to one chemotherapeutic agent may be sensitive to other agents. In clinical practice, infections due to strains of Neisseria that have become resistant to penicillin can usually be treated satisfactorily with one of the sulfonamide compounds Likewise, infections due to sulfonamide-resistant staphylococci frequently yield to penicillin.

The possibilities of this approach to the problem of drug-resistant tubercle bacilli are many, and provide the clinical investigator as well as the experimentalist with a challenge that is only excelled by

*Since preparation of this manuscript a preliminary report has been published on this subject See Karlson, A G, Pfuetze, K H, Carr, D T, Feldman, W H, and Hinshaw, H C · Proc Staff Meet, Mayo Clin 24, 85, 1949

the importance of the discovery of a substance that would by itself eliminate from the body all tubercle bacilli regardless of drug-resistant variants. The latter may be possible to achieve, but the unique anatomic characteristics of tuberculosis constitute a formidable obstacle to its realization.

Comment

No longer should infections by the tubercle bacillus be considered beyond the effective range of drug therapy. Evidence obtained during the past few years has established definitely that tuberculosis is vulnerable to the suppressive effects of drugs The future possibilities of discovering even more highly potent specific substances or combination of substances are promising Although streptomycin seems likely to become generally accepted as a useful drug in certain types of clinical tuberculosis, this substance falls short of the ideal tuberculochemotherapeutic agent. However, it is reassuring to recognize that streptomycin is not the first, nor it is likely to be the last, of specific antibacterial agents capable of exerting a favorable influence on a tuberculous infection.

When the complex character of the pathogenesis of tuberculosis is considered, it is understandable why a complete chemotherapeutic triumph over tuberculosis can be accomplished only by a substance capable of acting effectively under most formidable circumstances However, the factors to be reckoned with are not insurmountable, and should not deter further efforts to find more effective agents, nor do they necessarily imply that agents of even limited effectiveness are unworthy of extensive experimentation

Until more potent substances are available for chemotherapy in tuberculosis, workers concerned with the control of this disease should not neglect the application of other effective means of attacking the problem. Early diagnosis by mass roentgenographic surveys, BCG vaccination of those in the most susceptible age groups who are subjected to frequent exposure, segregation of patients likely to transmit tuberculosis to other persons, and adequate care with surgical and medical treatment in a sanatorium or hospital, are the indispensable features of an effective program of tuberculosis control. A highly effective chemotherapeutic agent to supplement such a program might well revolutionize the present concepts of tuberculosis for the physician, the surgeon, the patient, and society.

References

1 Alexander, H E, and Leidy, G · The present status of treatment for influenzal meningitis Am J Med 2, 457, 1947.
2 Baggenstoss, A H, Feldman, W H, and Hinshaw, H C.. Streptomycin in miliary tuberculosis, its effect on the pathological lesions of generalized tuberculosis in human beings Am Rev. Tuberc. 55, 54, 1947
3 Brown, H A, and Hinshaw, H C : Toxic reaction of streptomycin on the eighth nerve apparatus Proc Staff Meet, Mayo Clin 21, 347, 1946.
4 Cantani, A Quoted by Florey, H. W. (22).
5 Cook, E N, Greene, L F., and Hinshaw, H C · Streptomycin in the treatment of tuberculosis of the urinary tract Proc. Staff Meet, Mayo Clin 21, 277, 1946
5a Debré, R, Thiefry, S, and Brissaud, H E. La streptomycine appliquée au traitement de la méningite tuberculeuse et da tuberculose miliare chez l'enfant Paris, Masson et Cie, 1948.
6 Demerec, M Quoted by Selbie, F R. (53).
7 Domagk, G · Ein Beitrag zur Chemotherapie der bakteriellen Infektionen. Deutsche med Wchnschr. 1, 250, 1935
8 Feldman, W H Studies in chemotherapy of tuberculosis including use of streptomycin Tr & Stud, Coll Physicians, Philadelphia 14, 81, 1946
9 Feldman, W H "The chemotherapy of tuberculosis"—including the use of streptomycin· Harben lecture, 1946, The basic considerations of chemotherapy in tuberculosis J. Roy. Inst Pub. Health & Hyg 9, 267, 1946
10 Feldman, W H "The chemotherapy of tuberculosis"—including the use of streptomycin Harben lecture, 1946, An evaluation of the efficacy in tuberculosis of sulfonamides, sulfones and certain other substances J. Roy Inst Pub Health & Hyg 9, 297, 1946
11 Feldman, W H "The chemotherapy of tuberculosis"—including the use of streptomycin Harben lecture, 1946, The effect on tuberculosis of antagonistic substances of microbial origin with particular reference to streptomycin J Roy Inst Pub Health & Hyg 9, 343, 1946
12 Feldman, W H, and Hinshaw, H C Streptomycin in experimental tuberculosis, *in vivo* sensitivity to streptomycin of recently isolated strains of human tubercle bacilli and strains of bovine tubercle bacilli Am Rev. Tuberc 55, 428, 1947
13 Feldman, W H, and Hinshaw, H C Effects of streptomycin on experimental tuberculosis in guinea pigs A preliminary report Proc. Staff Meet, Mayo Clin 19, 593, 1944
14 Feldman, W H, Hinshaw, H C, and Karlson, A G Frequency of administration of streptomycin; its influence on results of treatment of tuberculosis in guinea pigs Am Rev Tuberc 55, 435, 1947
15 Feldman, W H, Hinshaw, H C, and Mann, F C. Streptomycin in experi-
16 tuber- ea pigs

of 4,2'-diaminophenyl-5'-thiazolysulfone (promizole). Am Rev. Tuberc. *60, 418, 1944*

17 Feldman, W H, Hinshaw, H C, and Moses, H. E: The effect of promin (sodium salt of *p,p'*-diaminodiphenyl sulfone *N,N'*-dextrose sulfonate) on experimental tuberculosis A preliminary report. Proc. Staff. Meet, Mayo Clin *15*, 695, 1940.

18 Feldman, W. H, Karlson, A. G, and Hinshaw, H. C.: Streptomycin-resistant tubercle bacilli; effects of resistance on therapeutic results in tuberculous guinea pigs Am. Rev. Tuberc. *57*, 162, 1948

19. Feldman, W H, Karlson, A G, and Hinshaw, H. C: Streptomycin in experimental tuberculosis The effects in guinea pigs following infection by intravenous inoculation Am Rev Tuberc *56*, 346, 1947.

20 Figi, F A, Hinshaw, H C, and Feldman, W H.· Treatment of tuberculosis of the larynx with streptomycin. Report of case. Proc. Staff. Meet, Mayo Clin *21*, 127, 1946

21 Finch, G H Streptomycin therapy in undulant fever. Am. J. Med. *2*, 485, 1947

22 Florey, H W The use of micro-organisms for therapeutic purposes Brit. M J *2*, 635, 1945

23 Foshay, L Treatment of tularemia with streptomycin. Am J. Med. *2*, 467, 1947

24 Golden, A *Personal communication*

25. Hart, P D Part I, Chemotherapy of tuberculosis, research during the past 100 years Part II, The sulphonamides and sulphones Brit. M. J. *2*, 805, 849, 1946

26 Heilman, D H, Heilman, F R, Hinshaw, H C, Nichols, D R, and Herrell, W E Streptomycin Absorption, diffusion, excretion and toxicity Am J M Sc *210*, 576, 1945

27 *Herrell, W E, and Heilman, F R Streptomycin, general considerations,* tests for bacterial sensitivity and methods of measurement of streptomycin in body fluids Am J Med *2*, 421, 1947

28 Hewitt, W L Treatment of urinary tract infections with streptomycin Am J Med *2*, 474, 1947

29 Hinshaw, H C, and Feldman, W H Streptomycin in treatment of clinical tuberculosis A preliminary report Proc. Staff Meet, Mayo Clin *20*, 313, 1945

30 Hinshaw, H C, Feldman, W H, and Pfuetze, K H. Treatment of tuberculosis with streptomycin, a summary of observations on one hundred cases J A M A. *132*, 778, 1946

31. Hinshaw, H. C., Feldman, W H, and Pfuetze, K H Streptomycin in treatment of clinical tuberculosis Am Rev Tuberc *54*, 191, 1946

32 Howes, E. L Topical use of streptomycin in wounds Am J Med *2*, 449, 1947

33 Hunter, T. H: Use of streptomycin in the treatment of bacterial endocarditis Am J. Med *2*, 436, 1947

34. Karlson, A G: Personal communication

35 Karlson, A G, and Feldman, W H · Resistance of tubercle bacilli to

streptomycin in guinea pigs after administration of the drug· The effect on response to treatment with streptomycin J. Bact *54*, 67, 1947.
36 Karlson, A. G, Feldman, W. H, and Hinshaw, H C. Persistance of resistance of tubercle bacilli to streptomycin during passage through guinea pigs Proc. Soc Exper. Biol & Med. *64*, 6, 1947.
37. Keefer, C. S, Blake, F. G, Lockwood, J S, Long, P H, Marshall, E. K, Jr, and Wood, W. B, Jr.: Streptomycin in the treatment of infections; a report of one thousand cases J A. M. A *132*, 4, 1946
38 Koch, R. Die Aetiologie der Tuberculose Berl klin Wchnschr *19*, 221, 1882
39 Lourie, E M A sketch of the history of chemotherapy Brit M Bull *4*, 243, 1946
40 McDermott, W Toxicity of streptomycin. Am J Med *2*, 491, 1947.
40a McDermott, W, Muschenheim, C, Hadley, S J, Bunn, P. A, and Gorman, R V.: Streptomycin in the treatment of tuberculosis in humans I Meningitis and generalized hematogenous tuberculosis Ann Int Med *27*, 769, 1947.
40b Medical Research Council, Streptomycin in Tuberculosis Trials Committee Streptomycin treatment of tuberculous meningitis Lancet *1*, 582, 1948
41. Meyer, K F The prevention of plague in the light of newer knowledge Ann New York Acad Sc *48*, 429, 1947
42 Milgram, L, Levitt, I, and Unna, M S: Promizole treatment of miliary tuberculosis; toxic effects on thyroid gland and maturation Am Rev Tuberc *55*, 144, 1947.
43 Molitor, H Pharmacology of streptomycin Bull New York Acad. Med. *23*, 196, 1947
44 Nichols, D R Personal communication
45 Olsen, A M Nebulization therapy in bronchiectasis; the use of penicillin and streptomycin aerosols J A M A *134*, 947, 1947
46 Oswald, E J, and Nielsen, J K Studies on the stability of streptomycin in solution Science *105*, 184, 1947.
47 Pasteur, L, and Joubert, J Carbon et septicémie Compt rend Acad d sc *85*, 101, 1877
48 Pyle, M M Relative numbers of resistant tubercle bacilli in sputa of patients before and during treatment with streptomycin Proc Staff Meet Mayo Clin *22*, 465, 1947
49 Rich, A R, and Follis, R H, Jr The inhibitory effect of sulfanilamide on the development of experimental tuberculosis in the guinea pig Bull. Johns Hopkins Hosp *62*, 77, 1938
50 Rist, N, Bloch, F, and Hamon, V: Action inhibitrice du sulfonamide et d'une sulfone sur la multiplication in vitro et in vivo du bacille tuberculeux aviaire Ann. Inst Pasteur. *64*, 203, 1940
51 Schatz, A, and Waksman, S A. Effect of streptomycin and other antibiotic substances upon Mycobacterium tuberculosis and related organisms Proc Soc Exper Biol & Med *57*, 244, 1944.
52 Schatz, A, Bugie, E, and Waksman, S A· Streptomycin, a substance ex-

hibiting antibiotic activity against gram-positive and gram-negative bacteria Proc Soc Exper Biol. & Med *55*, 66, 1944.
53 Selbie, F. R · Microbial resistance to chemotherapeutic drugs. Brit M. Bull *4*, 267, 1946.
54 Smith, H. V., and Daniel, P : Some clinical and pathological aspects of tuberculosis of the central nervous system Tubercle *28*, 64, 1947
55. Smith, M I., and McClosky, W T : The chemotherapeutic action of streptomycin and promin in experimental tuberculosis. Pub. Health Rep. *60*, 1129, 1945
56 Waksman, S A · Microbial Antagonisms and Antibiotic Substances Rev ed, pp. 191–200 New York, The Commonwealth Fund, 1947.
57 Youmans, G P Personal communication.
58 Youmans, G P : The effect of streptomycin in vitro on M. tuberculosis var. hominis Quart Bull, Northwestern Univ M School. *19*, 207, 1945.
59 Youmans, G P, and Karlson, A G · Streptomycin sensitivity of tubercle bacilli, studies on recently isolated tubercle bacilli and the development of resistance to streptomycin in vivo Am Rev Tuberc. *55*, 529, 1947.
60 Youmans, G. P, and McCarter, J C · Streptomycin in experimental tuberculosis, its effect on tuberculous infections in mice produced by M tuberculosis var hominis Am Rev Tuberc *52*, 432, 1945.
61. Youmans, G P, Williston, E H, Feldman, W H, and Hinshaw, H. C.: Increase in resistance of tubercle bacilli to streptomycin A preliminary report Proc Staff Meet, Mayo Clin *21*, 126, 1946.

Histoplasmosis

HENRY PINKERTON
St Louis University School of Medicine, St Louis, Missouri

Introduction

Histoplasmosis, also called histoplasmosis of Darling, cytomycosis of Darling, and reticulo-endothelial cytomycosis may be defined as an acute, subacute, or chronic systemic infection caused by the fungus Histoplasma capsulatum

Perhaps the most interesting feature of the disease is the protean nature of its clinical manifestations, which may suggest primary disease of practically any organ. A patient with histoplasmosis may be first seen by the pediatrician, the internist, the hematologist, the dermatologist, the otolaryngologist, the cardiologist, the phthisiologist, the orthopedic surgeon, or by any other specialist. Present evidence indicates, however, that almost invariably the infection eventually becomes generalized. The involvement of a particular organ so that it dominates the picture may depend to some extent on the route of inoculation, but is probably to a large extent a matter of chance

In the early stages, the patient may be afebrile, and free from evidence of generalized infection The only complaint may be, for example, of an ulcer in the mouth. Sooner or later, however, signs of systemic involvement appear, such as fever, weight loss, anemia, leukopenia, splenomegaly, hepatomegaly, and enlargement of the superficial lymph nodes Death may occur in a few weeks or after a period of several years The mortality of clinically recognized cases has hitherto been practically 100 per cent

In recent years it has become clear that histoplasmosis is not a particularly rare infection, and that it can be diagnosed and properly studied only if it is considered in the differential diagnosis of many clinical syndromes. This has made the disease one of peculiar interest to both clinicians and pathologists The literature on the

subject consists mainly of case reports, with reviews of previously reported cases, and attempts to fit the new cases into the picture. In this way, the clinical picture of histoplasmosis, which is one full of vagaries, is being gradually crystallized, but it is probable that all of the forms which the disease may assume have not yet been described

Diagnosis depends on the demonstration, by direct microscopic examination, by culture methods, or by animal inoculation, that the causative organism, H capsulatum, is present.

History

In 1905, Darling, while studying leishmaniasis in the Panama Canal Zone, found an unusual pathologic picture in a 27 year old Negro from Martinique. Splenomegaly and fever were the clinical features. Postmortem study showed "pseudotubercles in the lungs, focal necrosis in the liver, spleen, and lymph nodes," and many intracellular organisms approximately the size of the aflagellate intracellular forms of Leishmania donovani Darling noted, however, that the organism differed from Leishmania in several ways, notably in the presence of a well-defined capsule He considered it to be a previously unrecognized protozoon, and named it Histoplasma capsulatum. This first case of histoplasmosis was reported in 1906 (16).

In the same year, Strong (70), while working in the Philippines, described a similar organism found in curettings from an abscess in the chest wall of a 35 year old Filipino woman This lesion healed following the application of antiseptic dressings. Parsons and Zarafonetis (57) quote Strong as now believing that "the case which he described was one of those unusual instances of the infection of man with Cryptococcus farciminosus." (51)

In 1907, Darling (17,18) reported two more fatal cases, clinically similar to the first, and on postmortem study showing morphologically indentical organisms in the tissues He realized that this newly discovered organism, like Leishmania, was located chiefly in the cells of the reticulo-endothelial system On the basis of his three cases, he stated that the outstanding clinical features of the disease were irregular fever, emaciation, anemia, leukopenia, and splenomegaly. This concept, by and large, still holds true today.

Da Rocha-Lima (19,20), in 1912, reviewed the sections from Darling's cases, and expressed the opinion that the organism was a yeastlike fungus, rather than a protozoon. He believed further that it was closely related to *Cryptococcus farciminosus*, the etiologic agent of infectious lymphangitis in horses, which is a rare cause of human infection.

In 1924, Riehl (63) reported granulomatous lesions of 7 years' duration in a white man, caused by a yeastlike organism; the patient eventually died. Moore and Jorstad (49) believe this case to be one of histoplasmosis, basing their belief on the typical appearance of the organisms in the illustrations accompanying the report

The fifth case of histoplasmosis, and the first to be described in this country was that reported by Watson and Riley (78) in 1926. This case originated in Minnesota, a region where the disease is now believed to be particularly uncommon, and occurred in an adult Autopsy showed generalized involvement, similar to that originally found by Darling Watson (79) emphasized the tendency of the organism to grow in cells of reticulo-endothelial origin, and made cytologic studies of considerable interest

In the same year, Phelps and Mallory (58) and Wade (77) reported cases of what was undoubtedly histoplasmosis In 1931, Crumrine and Kessel (15) published a case with outstanding involvement of the gastrointestinal tract, and the next year Muller (50) reported a typical case of generalized infection in a 7 year old Javanese boy

In 1933, Hansmann and Schenken (30) and De Monbreun (22) *succeeded in cultivating the organism* The case reported by Hansmann and Shenken showed involvement of the skin and buccal mucosa The organism which they cultivated was regarded by them as a member of the genus Sepedonium, but later studies showed it *to be Histoplasma*

De Monbreun (22) cultivated Histoplasma from the blood stream during life, and on autopsy from the spleen of a 5 month old infant with a generalized infection The cultivation of the organism brought *definite proof that it was a fungus rather than a protozoon*, confirming the opinion expressed by Da Rocha-Lima in 1912, on purely morphologic grounds De Monbreun showed definitely that the organism was distinct from Cryptococcus farciminosus.

Incidence

In 1940, Meleney (44) was able to summarize 32 cases of histoplasmosis, the majority of which had occurred during the preceding 15 months. Since that time, the number of reported cases has increased each year, and it is no longer considered a duty to report cases unless they add something new to the picture of the disease. In 1945, Parsons and Zarafonetis (57) reviewed 71 cases, reported 7 new cases, and referred to 3 others, making a total of 81 cases

There is some difference of opinion as to whether the rapid increase in number of cases reported represents a true increase in incidence Several cases have been discovered by reviewing autopsy material from hospital files, one 13 years and another 21 years after the autopsy was performed In some of these cases, organisms have been present in such large numbers that it is difficult to see how they could have been overlooked However, when one considers the fact that in some cases the organisms can be found only in one organ, it seems probable that many cases were overlooked in the past Possibly there has been, in recent years, both an increased recognition of the disease and a true increase in its incidence

Histoplasmosis is a disease of all ages, and the possibility of congenital infection has been suggested (36) Parsons and Zarafonetis (57) have tabulated the age distribution of 60 patients whose ages were known The incidence was highest during the first year of life (11 cases), then fell sharply until it reached another peak in the fifth, sixth, and seventh decades Symptoms were first noted during the second month of life in 3 cases, and possibly during the first month in 1 case

The same authors found that of 65 patients of known sex 51 were males and 14 females Up to the age of 10, however, there were 8 males and 8 females, while of the 49 patients over 10 years of age, 43 were males A similar predilection for males is seen in actinomycosis

Of 63 known cases, there were 51 white patients, 7 Negroes, 2 mulattoes, 1 Javanese, 1 Chinese, and 1 Honduran

Of 30 patients above 12 years of age whose occupations were known, 10 were farmers, 4 laborers, 2 bartenders, and 1 each of the

following: baker, carpenter, janitor, lawyer, mine manager, printer, salesman, teacher, soldier, steelworker, student, and teamster.

GEOGRAPHIC DISTRIBUTION

Here, again, there is doubt whether the incidence of the disease, as reported from different regions, can be taken as an index of its true distribution The distribution of the 78 cases reviewed by Parsons and Zarafonetis is: 56 cases in the United States, 3 in the Panama Canal Zone, 3 in Brazil (76), 2 in Argentina (5), and 1 each in Austria, British Honduras, East Java, England, Mexico, the Philippines, and Southern Rhodesia. In the United States 1 case has occurred in each of the following states: Florida, Iowa, Louisiana, Maryland, Minnesota, Wisconsin, Mississippi, New York, North Carolina, Ohio, and Oklahoma; 2 cases each in Kentucky, Texas, Virginia, and Washington, D. C.; 3 cases in Indiana; 4 cases in Illinois, 7 cases in Tennessee; and 10 cases each in Missouri and Michigan

These figures suggest that the disease is widely distributed in the temperate, subtropical, and tropical countries of the world, and that there may be a particularly high incidence in the midwestern United States

Etiology

Nomenclature. The etiologic agent of histoplasmosis is a fungus, which, in spite of its intracellular location in infected tissues, is readily cultivated in a variety of mediums Moore (46,47), on the basis of careful morphologic studies, thought that the strain isolated by Hansmann and Schenken showed features which distinguished it from the strain isolated by De Monbreun, and named the organisms Histoplasma pyriforme and Histoplasma capsulatum, respectively. He believed that the occurrence of large forms in the strain isolated by Hansmann and Schenken was a significant feature However, Henderson, Pinkerton, and Moore (32), and others have observed considerable variation in the size of the organism in different organs from the same case, or even in single microscopic fields. On the whole, evidence for the existence of different species seems to be insufficient as yet, and the name Histoplasma capsulatum, originally

used by Darling, seems most suitable for all recognized strains of the organism.

The taxonomic status of the organism is still somewhat uncertain. Conant (14) placed it in the family Moniliaceae of the Fungi imperfecti, while Ciferri, Redaelli, and Visocchi (12) have created the family Histoplasmaceae and the superfamily Atelosaccharomycetaceae for the genus.

Pathogenicity The organism is pathogenic for a variety of lower animals Hansmann and Schenken (31) succeeded in infecting dogs and cats with their strain, and De Monbreun (22) infected the dog, the monkey, and the mouse. Guinea pigs (52), rats (31), and rabbits (31) are also susceptible In all cases, lesions resembling those in man have been produced in lower animals, and organisms were numerous in these lesions. This fact, together with the presence of huge numbers of organisms in infected human tissues, leaves no doubt concerning the etiologic relationship of the organism to the disease By varying the dosage and the route of inoculation, acute, subacute, or chronic, localized infections may be produced in animals (71)

Morphology

In tissues, the organisms most commonly and most characteristically appear as rounded, doubly contoured yeastlike bodies, closely packed within cells which are largely, but not entirely, of reticuloendothelial origin (see section on Pathology). Over large areas, the organisms may be so numerous that the tissue presents a "honeycombed" appearance Budding is occasionally seen, and oval forms are not uncommon The average diameter is 2 5 to 3 5 μ, but forms as large as erythrocytes are occasionally seen

A characteristic feature is the presence of a clear, nonstaining, refractile capsule which forms about $^1/_6$ the total diameter (24,34). Under certain conditions, particularly in sections stained with Giemsa's stain, the capsule may be brilliantly illuminated by using polarized light

A definite nucleus is not seen The substance of the organism within the capsule is basophilic, and somewhat granular and vacuolated, often the basophilic chromatin is concentrated eccentrically, so that a crescentic or signet ring appearance is produced Occasion-

ally, the central portion of the organism is uniformly stained The appearance of the organism may be seen in Figure 1.

Rarely, mycelial forms are seen in infected tissues The yeastlike form, however, appears to be the characteristic pathogenic phase. and the appearance of mycelia may indicate postmortem growth

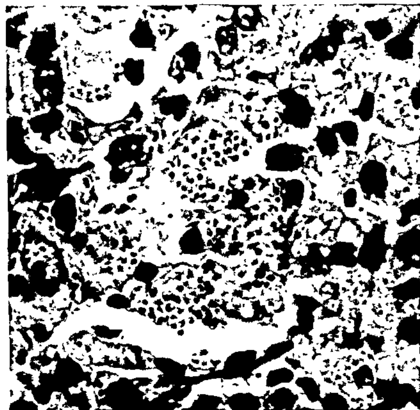


Fig 1 Histoplasma capsulatum in adrenal epithelial cells adjacent to an area of caseous necrosis Note the unstained capsule and the presence of signet ring forms In a few organisms the central portion is uniformly stained

Organisms of roughly similar size and shape found in tissues which must be differentiated from Histoplasma (60) are (*1*) aflagellate forms of Leishmania and Trypanosoma cruzi, (*2*) Toxoplasma, (*3*) Sarcocystis, and (*4*) Encephalitozoon Leishmania and Trypano-

used by Darling, seems most suitable for all recognized strains of the organism.

The taxonomic status of the organism is still somewhat uncertain. Conant (14) placed it in the family Moniliaceae of the Fungi imperfecti, while Ciferri, Redaelli, and Visocchi (12) have created the family Histoplasmaceae and the superfamily Atelosaccharomycetaceae for the genus.

Pathogenicity The organism is pathogenic for a variety of lower animals. Hansmann and Schenken (31) succeeded in infecting dogs and cats with their strain, and De Monbreun (22) infected the dog, the monkey, and the mouse Guinea pigs (52), rats (31), and rabbits (31) are also susceptible In all cases, lesions resembling those in man have been produced in lower animals, and organisms were numerous in these lesions. This fact, together with the presence of huge numbers of organisms in infected human tissues, leaves no doubt concerning the etiologic relationship of the organism to the disease By varying the dosage and the route of inoculation, acute, subacute, or chronic, localized infections may be produced in animals (71)

Morphology

In tissues, the organisms most commonly and most characteristically appear as rounded, doubly contoured yeastlike bodies, closely packed within cells which are largely, but not entirely, of reticulo-endothelial origin (see section on Pathology) Over large areas, the organisms may be so numerous that the tissue presents a "honey-combed" appearance Budding is occasionally seen, and oval forms are not uncommon The average diameter is 2 5 to 3 5 μ, but forms as large as erythrocytes are occasionally seen.

A characteristic feature is the presence of a clear, nonstaining, refractile capsule which forms about $^1/_6$ the total diameter (24,34). Under certain conditions, particularly in sections stained with Giemsa's stain, the capsule may be brilliantly illuminated by using polarized light.

A definite nucleus is not seen The substance of the organism within the capsule is basophilic, and somewhat granular and vacuolated; often the basophilic chromatin is concentrated eccentrically, so that a crescentic or signet ring appearance is produced Occasion-

ally, the central portion of the organism is uniformly stained. The appearance of the organism may be seen in Figure 1.

Rarely, mycelial forms are seen in infected tissues. The yeastlike form, however, appears to be the characteristic pathogenic phase, and the appearance of mycelia may indicate postmortem growth

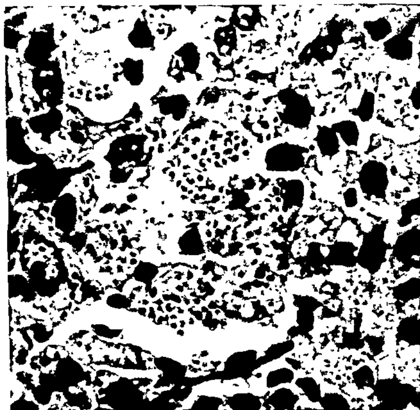


Fig 1 Histoplasma capsulatum in adrenal epithelial cells adjacent to an area of caseous necrosis Note the unstained capsule and the presence of signet ring forms In a few organisms the central portion is uniformly stained.

Organisms of roughly similar size and shape found in tissues which must be differentiated from Histoplasma (60) are (*1*) aflagellate forms of Leishmania and Trypanosoma cruzi, (*2*) Toxoplasma, (*3*) Sarcocystis, and (*4*) Encephalitozoon Leishmania and Trypano-

used by Darling, seems most suitable for all recognized strains of the organism.

The taxonomic status of the organism is still somewhat uncertain. Conant (14) placed it in the family Moniliaceae of the Fungi imperfecti, while Ciferri, Redaelli, and Visocchi (12) have created the family Histoplasmaceae and the superfamily Atelosaccharomycetaceae for the genus

Pathogenicity The organism is pathogenic for a variety of lower animals Hansmann and Schenken (31) succeeded in infecting dogs and cats with their strain, and De Monbreun (22) infected the dog, the monkey, and the mouse Guinea pigs (52), rats (31), and rabbits (31) are also susceptible In all cases, lesions resembling those in man have been produced in lower animals, and organisms were numerous in these lesions This fact, together with the presence of huge numbers of organisms in infected human tissues, leaves no doubt concerning the etiologic relationship of the organism to the disease By varying the dosage and the route of inoculation, acute, subacute, or chronic, localized infections may be produced in animals (71)

Morphology

In tissues, the organisms most commonly and most characteristically appear as rounded, doubly contoured yeastlike bodies, closely packed within cells which are largely, but not entirely, of reticulo-endothelial origin (see section on Pathology) Over large areas, the organisms may be so numerous that the tissue presents a "honeycombed" appearance Budding is occasionally seen, and oval forms are not uncommon. The average diameter is 2 5 to 3 5 μ, but forms as large as erythrocytes are occasionally seen.

A characteristic feature is the presence of a clear, nonstaining, refractile capsule which forms about $^1/_6$ the total diameter (24,34). Under certain conditions, particularly in sections stained with Giemsa's stain, the capsule may be brilliantly illuminated by using polarized light

A definite nucleus is not seen. The substance of the organism within the capsule is basophilic, and somewhat granular and vacuolated; often the basophilic chromatin is concentrated eccentrically, so that a crescentic or signet ring appearance is produced Occasion-

artificial mediums, including Sabouraud's maltose or glucose agar, dextrose blood agar, and potato-dextrose medium. It is primarily aerobic, but may grow under partial anaerobiasis. It usually grows rather slowly, colonies appearing in 3 to 10 days, and reaching a diameter of 2 to 3 cm. by the fifteenth day. Moore (49) has grown Histoplasma on the chorioallantoic membrane of the egg. The membrane was inoculated through a window cut in the shell on the twelfth day of incubation Under these conditions, conversion of a 7 year old culture from the saprophytic (filamentous) to the parasitic (yeastlike) form was observed. A characteristic cellular reaction, including the formation of giant cells, was noted in the membrane.

Biochemical Characteristics The biochemical and immunologic properties of the organism have been studied by Scheff (66). The nutritional requirements are rather simple. Glucose was utilized by the organism, but was not essential to its growth Nitrogen was used most readily in the form of sodium aspartate or asparagine, but ammonium sulfate alone was adequate for slow growth, serving as a source for both nitrogen and carbon The basic medium used consisted of asparagine, glucose, liver extract, potassium dihydrogen phosphate (KH_2PO_4), magnesium sulfate ($MgSO_4$), calcium chloride ($CaCl_2$), ammonium sulfate ($(NH_4)_2SO_4$), zinc sulfate ($ZnSO_4$), ferric chloride ($FeCl_3$), manganese chloride ($MnCl_2$), copper sulfate ($CuSO_4$), boric acid (H_3BO_3), and potassium iodide (KI). The organism, like other fungi, shows a low metabolic rate. A polysaccharide and a protein fraction were isolated from the mycelian form Even after infection of long duration, the humoral antibody response was slight, but cutaneous sensitivity was pronounced. With active immunization, antibody formation was more marked and skin sensitivity less marked Scheff concludes that the occurrence of skin sensitivity depends on the presence of living organisms in the body, while a high antibody titer occurs only with high antigen concentration

Epidemiology

Epidemiologic problems in histoplasmosis are to a large extent unsolved Apparently the disease is never *epidemic in nature* The organism is so resistant that it may live for long periods in the

soma cruzi are readily identified by the presence of a rod-shaped kinetoplast, in addition to the nucleus. Toxoplasma has a definite nucleus which stains differentially from the cytoplasm Sarcocystis is a larger organism, more falciform in shape, and occurring in large cysts Encephalitozoon is usually smaller, often without a nucleus, and contains clear areas This organism perhaps most closely resembles Histoplasma, but none of the four organisms mentioned above has the definite refractile capsule so characteristic of Histoplasma

In cultures with a high protein content, such as blood or serum agar, inoculation of infected tissue containing the yeastlike form results in the continued growth of the yeastlike form if the cultures are maintained at 37 C. At room temperature, De Monbreun (22) found that the mycelial form appeared in all cultures. Conant (14) was able to convert the mycelial form into the yeastlike form by the use of sealed, blood agar slants kept at 37 C Negroni (52) found that incubation in weak solutions of sodium hydroxide or sodium borate at 37 C resulted in the conversion of the mycelial into the yeastlike form. *The mycelial form is fully pathogenic for animals, but changes to the rounded, budding form on reaching living animal tissue*

In cultures, the septate hyphae are 1 to 5 μ in diameter The chlamydospores are 3 to 10 μ or larger in diameter, and tuberculate or spiny The exact function of these large, tuberculate spherical cells is not clear (46)

Staining Characteristics The organism usually is recognized quite readily in sections stained with hematoxylin and eosin or eosin–methylene blue Parsons and Zarafonetis (57, case E), however, found many recognizable organisms in sections of the tricuspid valve with attached vegetations, stained with Giemsa's stain, after failing to find any organisms in similar sections stained with hematoxylin and eosin The body of the organism is faintly basophilic, with unstained areas of irregular shape, while the capsule remains unstained In smear preparations of circulating blood or bone marrow, Giemsa's stain or Wright's stain is satisfactory In the author's experience, special stains for yeasts have not been particularly helpful The organism is weakly gram positive and slightly acid fast (55).

Culture Characteristics Histoplasma grows readily on many

of Histoplasma capsulatum. Again healing followed radiotherapy, and the patient thereafter remained well and was alive 5 years later. The patient's husband had a similar ulcer of the tongue which antedated his wife's by several months. He died 4 years after the onset, without biopsy or autopsy, and a definite diagnosis was not made.

The fact that infection has appeared to be primary in and confined to the lungs in several patients has suggested the respiratory tract as a possible portal of entry Henderson *et al* (32), however, were unable to infect dogs or guinea pigs by intratracheal injection of cultures which readily caused infection by other routes.

In several infants there has been otitis media, with Histoplasma organisms present in the discharge from the ear. This has suggested the ear as a primary focus, with later extension to other organs.

Since intracellular yeasts are common in insects, the possibility of insect transmission of histoplasmosis should be kept in mind.

Pathology

A wide variety of pathologic lesions may be found at autopsy, depending somewhat on the route of infection, but to a large extent on the localization and progress of the lesions in the various organs Thus, the immediate cause of death may be perforation of an intestinal ulcer, extensive pulmonary involvement, vegetative endocarditis with embolic phenomena, adrenal insufficiency due to bilateral adrenal necrosis, cachexia, or any one of a large number of other causes

The *skin*, in cases of the cutaneous type, may show extensive nodules or ulceration, while in the systemic type of infection papular or petechial eruptions are not uncommon Ulcers may be present on mucocutaneous surfaces, or in the nasal or oral cavities, larynx, or pharynx

Localized or generalized *lymph node enlargement*, involving superficial or deep groups of nodes, is almost invariably present. Localized, opaque, caseous nodules, ranging from miliary size to 2 cm in diameter, are often found in various organs, and the pathology, as indicated by Darling in his original description, generally resembles that of tuberculosis.

As a rule the *heart* remains essentially normal, except in cases with vegetative mycotic endocarditis. In one case (39), however,

The fact that experimental laboratory infection has not occurred among the people who have worked with the organism in animals or in cultures would suggest that the majority of individuals are relatively refractory to the infection. Nor do we know what are the factors which make human beings susceptible to the infection.

There can be no doubt that "reservoirs" of infection exist in lower animals. Spontaneous infection in the dog was first reported by De Monbreun (23), and later by Thuringer (72), Callahan (10), Parsons and Everett (56) and Tomlinson and Grocott (73). Organisms identical with or closely related to Histoplasma capsulatum have been found in ferrets (40) and in mice. Presumably infection in lower animals plays some role in the epidemiology of the disease in man, but contact with sick animals has not been mentioned in the literature. It is perhaps significant that the case reported by Kuzma and Schuster (39) occurred in a dog breeder.

The frequent occurrence of the infection in young babies is a puzzling fact, possibly indicating the widespread occurrence of the organism in nature The situation may be analogous to that in infection with the tubercle bacillus. Such a theory would harmonize well with the view taken by certain recent workers, who have attempted to explain certain prevalent types of pulmonary calcification as representing healed, asymptomatic Histoplasma infections (page 224)

Even concerning the portal of entry there is little definite information. Experimentally, infection has been transmitted to dogs by the oral route and by parenteral injection, the character of the disease depending on the dosage and route of inoculation

The oral and the cutaneous routes seem to be the most likely ones in man The former is suggested by the frequent occurrence of lesions in the mouth, larynx, and pharynx, which may be present for many months before fever develops, and by the occurrence of intestinal ulcers and diarrhea as an apparently primary lesion in certain cases Cutaneous and mucocutaneous lesions, including penile involvement, may likewise be present for a long time before generalized involvement occurs It is noteworthy that in one of the patients (case H) reported by Parsons and Zarafonetis (57) there is evidence for direct contact infection The patient was a 68 year old woman with an ulcer of the tongue which healed after radiotherapy A year later two ulcers appeared, and biopsy showed the presence

usually erode only the mucosa and submucosa. Perforation has been reported in only one instance Practically all regions of the gastrointestinal tract have been involved in isolated cases. The *adrenals* are quite often grossly affected, showing caseous necrosis greatly resembling that caused by the tubercle bacillus. Other organs less commonly involved are the kidneys, pancreas, thymus, brain, and prostate

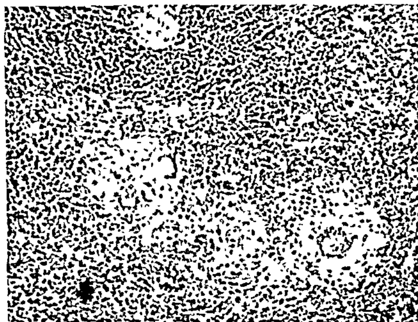


Fig 3 Noncaseous, tuberclelike lesions with central giant cells in the spleen No Histoplasma organisms were found in these lesions

Microscopically, the picture varies considerably, depending on the extent of gross involvement Commonly, organisms are present in the involved areas in such numbers that the tissue is recognized with difficulty Organisms are usually absent in the areas of actual necrosis, but at the edges of the necrotic lesions the tissue may be "honeycombed" with rounded, encapsulated yeastlike bodies

In the lungs, the alveolar walls are often so distended with phagocytic cells laden with Histoplasma that the alveoli are partially obliterated Even the moderate-sized arteries may be almost com-

marked interstitial myocarditis with organisms in macrophages was noted.

The *lungs* have shown specific involvement by Histoplasma in at least 11 cases. The picture is that of consolidation, caseation, and cavitation, resembling grossly the lesion of chronic tuberculosis In a few cases, tuberculous lesions have been found along with those of histoplasmosis Secondary bacterial bronchopneumonia may develop as a terminal event.

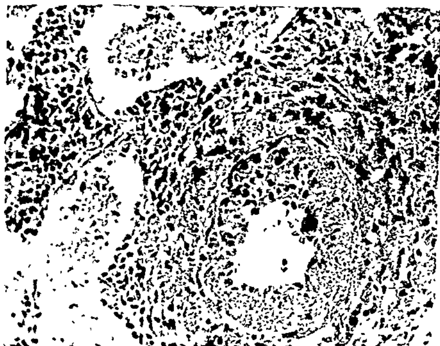


Fig 2 Invasion of the wall of an artery in the lung by Histoplasma The organisms, which appear as small rounded dots, are intracellular in position

The *spleen* is enlarged in about half of the cases, and localized, tuberclelike lesions of miliary size or larger are often seen grossly The *liver*, too, is apt to be enlarged, and gross lesions may be evident. Involvement of liver and spleen is characteristic of the generalized type of the disease About a third of the cases have shown ulcerative lesions in the *ileum*, in the *large intestine*, or in both The ulcers vary from pinpoint size to 2 cm or more in diameter, and

usually erode only the mucosa and submucosa Perforation has been reported in only one instance Practically all regions of the gastrointestinal tract have been involved in isolated cases The *adrenals* are quite often grossly affected, showing caseous necrosis greatly resembling that caused by the tubercle bacillus. Other organs less commonly involved are the kidneys, pancreas, thymus, brain, and prostate

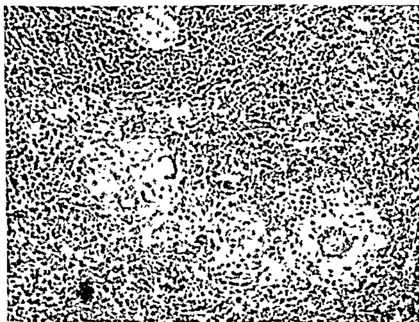


Fig 3 Noncaseous, tuberclelike lesions with central giant cells in the spleen No Histoplasma organisms were found in these lesions

Microscopically, the picture varies considerably, depending on the extent of gross involvement Commonly, organisms are present in the involved areas in such numbers that the tissue is recognized with difficulty Organisms are usually absent in the areas of actual necrosis, but at the edges of the necrotic lesions the tissue may be "honeycombed" with rounded, encapsulated yeastlike bodies

In the lungs, the alveolar walls are often so distended with phagocytic cells laden with Histoplasma that the alveoli are partially obliterated Even the moderate-sized arteries may be almost com-

marked interstitial myocarditis with organisms in macrophages was noted

The *lungs* have shown specific involvement by Histoplasma in at least 11 cases The picture is that of consolidation, caseation, and cavitation, resembling grossly the lesion of chronic tuberculosis. In a few cases, tuberculous lesions have been found along with those of histoplasmosis Secondary bacterial bronchopneumonia may develop as a terminal event

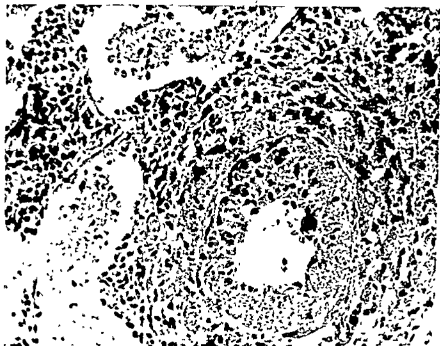


Fig 2 Invasion of the wall of an artery in the lung by Histoplasma The organisms, which appear as small rounded dots, are intracellular in position

The *spleen* is enlarged in about half of the cases, and localized, tuberclelike lesions of miliary size or larger are often seen grossly The *liver*, too, is apt to be enlarged, and gross lesions may be evident *Involvement of liver and spleen is characteristic of the generalized type of the disease* About a third of the cases have shown ulcerative lesions in the *ileum*, in the *large intestine*, or in both The ulcers vary from pinpoint size to 2 cm or more in diameter, and

cases (Figs 3 and 4). Frequently, these tuberclelike lesions contain no organisms. The giant cells are often vacuolated, as though they contained the empty capsules of organisms. This giant-cell reaction apparently represents a relatively good state of resistance. In areas where the tissue is almost unrecognizable because great numbers of organisms are present, there is frequently an absence of inflammatory cell response.

The bone marrow is frequently involved, and for this reason sternal puncture is an important diagnostic procedure Grossly, the bone marrow may be hyperplastic, in adults, the normal, fatty appearance of the marrow in the femur may change to that of a red or gray tissue, suggesting the picture seen in leukemia or pernicious anemia. Microscopically, large pale cells of reticulo endothelial origin may be so numerous that blood-forming cells are practically absent. These cells are uniformly filled with Histoplasma (see Fig 5 on page 212)

CLINICAL PATHOLOGY

Blood The degree to which the bone marrow is involved by the parasites largely governs the blood picture, although the possibility of a toxic effect without the actual presence of the fungus cannot be excluded In the early stages, particularly those with cutaneous, naso-oral, or pharyngeal involvement, the blood picture may be normal In patients with evidence of systemic involvement (fever, emaciation, splenomegaly, hepatomegaly, and lymph node enlargement) a variety of hematologic changes may be present. Wide variations are found in any one series of cases, but the most common abnormalities are moderate to severe anemia with leukopenia

The *red cell count* has been below 4,000,000 in about 75 per cent of the cases on which data are available, and between 2,000,000 and 3,000,000 in about 25 per cent Counts as low as 1,500,000 and 1,000,000 have been recorded The *hemoglobin* is reduced in proportion to the lowered cell count The anemia is usually of the hypochromic type, and either normocytic or microcytic

The *white cell count* is also variable In about one-half of the cases, repeated counts have been within normal limits, in about one-third, there has been persistent leukopenia (lowest count 1,400), and in about one-sixth (disregarding 3 cases of possible leukemia), the count has been consistently but only moderately above normal (normal range, 5,000 to 10,000 white cells per cubic millimeter).

pletely replaced by organisms (Fig 2). The picture tends to be that of interstitial pneumonitis

As noted by many observers (1,35,79,64), the reticuloendothelial cells are conspicuously parasitized, a fact which led Humphrey (35) to apply the term "reticulo-endothelial cytomycosis" to the disease. However, epithelial cells are also involved, particularly the intestinal epithelium, the squamous epithelium of mucous surfaces, liver cord cells, and adrenal epithelial cells (Fig 1).

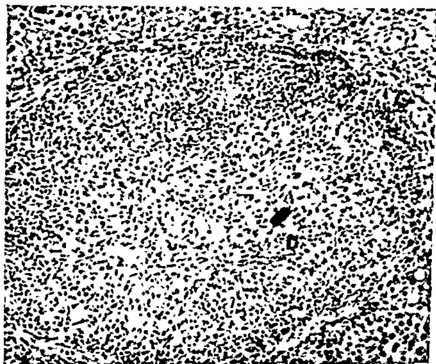


Fig 4 A large, pseudotubercle in the liver, composed of epithelioid cells with a peripheral collar of lymphocytes No giant cells were present, and Histoplasma organisms were not found

In cases of extensive generalized involvement, the spleen, liver, lung, lymph nodes, and bone marrow may contain several times as many Histoplasma organisms as they do tissue cells

In some cases, however, organisms are present only in localized areas in one or more organs Noncaseous tuberclelike lesions, with or without central giant cells, may be present in the organs of such

The signs and symptoms are pain in the ear, deafness, tinnitus, and vertigo

In several cases, there has also been involvement of the aural canal These cases are probably more closely related to the cutaneous type One patient was treated for a fungous infection of the external auditory canal shortly before the onset of systemic histoplasmosis.

Ocular Type. This appears to be rare, the only reported instance being that of Reid and co-workers, who described small, white, irregular tuberclelike areas, surrounded by hemorrhage, in the ocular fundi of their case. Through the courtesy of the Army Institute of Pathology, the author was permitted to study sections of an enucleated eye with extensive involvement by granulomatous lesions containing fungi which probably were Histoplasma

Generalized Type. Under this heading should be included those cases which, at the time of study, show evidence of systemic involvement without cutaneous, naso-oral, or other externally visible lesions, and without localized signs, such as severe diarrhea, pulmonary consolidation, or cardiac murmurs The onset is usually insidious, the patient may complain of having been in poor health for several weeks or months

The actual onset of illness is characterized by asthenia, anorexia, and a moderate, irregular fever At first, the temperature rises only in the afternoon or evening; later, the fever is more sustained In the majority of cases, however, the temperature does not exceed 102 5 F Marked loss of weight occurs after a few weeks, and the liver and spleen usually become palpable Localized or generalized enlargement of the superficial lymph nodes often develops at this time (Figs 6 and 7) There may be epigastric or lower abdominal pain, or generalized muscle and joint pains Headache is sometimes present There may be hoarseness and pharyngeal infection, even in the absence of any definite ulceration Diarrhea and vomiting may occur separately, or together.

The pulse rate is about proportional to the temperature. The respirations are not markedly increased unless pulmonary involvement develops The blood picture may remain normal, but there is usually some degree of anemia, and moderate leukopenia is not uncommon

After this picture of systemic involvement has become established there may be brief remissions of one or two weeks, but the general

Naso-oral Type. This may be a continuation of the mucocutaneous type, or lesions may occur in the mouth, nose, or throat without other evidence of infection.

From the point of view of the otorhinolaryngologist, as emphasized by Moore and Jorstad (49), histoplasmosis may simulate carcinoma, otitis media, rhinoscleroma, laryngeal tuberculosis, aleukemic leukemia, lymphoma, leishmaniasis, noma, blastomycosis, syphilis, nasopharyngitis, and laryngitis. The tongue has been involved in at least 22 cases, and in several instances a clinical diagnosis of carcinoma of the tongue was made

In 4 cases, there were nasal lesions in addition to those of the mucous membranes of the mouth. Perforation of the nasal septum occurred in 3 cases

The oral lesions have been discussed by Levy (42), and by Moore and Jorstad (49). The lesions, which may be single but are more often multiple, begin as verrucous, or indurated and raised nodules on the tongue, gums, or lips. Eventually they ulcerate, but the edges remain thickened, so that the picture closely resembles that of carcinoma The hard and soft palates, tonsils, epiglottis, and uvula may become involved, and in the later stages the larynx may become affected

In addition to these localized lesions which have been found in practically every portion of the naso-oral cavity, there may be diffuse involvement, with a grayish exudate on a hyperemic base

Laryngeal and Pharyngeal Involvement Usually, both larynx and pharynx are involved together (21). Granulomatous, ulcerated, or diffuse exudative lesions have been found in each in at least 10 cases

Moore and Jorstad have reviewed 22 cases with lesions of the ear, nose, and throat, in various combinations Aphonia, dysphonia, dysphagia, cough, dyspnea, and soreness of the throat are common symptoms

Otitic Type. The typical clinical picture of otitis media was present in 5 cases Such cases suggest middle ear infection as a primary lesion, with an epidemiology similar to that of other types of otitis media These lesions usually become generalized Frequently, there is spontaneous perforation of the ear drum and in at least 1 case, Histoplasma has been found in the purulent discharge

course is progressively downhill (7,9,13,29,68). Death is caused by cachexia and terminal bronchopneumonia unless fatal complications develop in some other organ.

Histoplasmosis Associated with Leukemia. The enlargement of the superficial lymph nodes, with splenomegaly and hepatomegaly, and the abnormalities in the blood picture described above, have often led to a tentative diagnosis of leukemia In 3 cases, the diagnosis of leukemia was apparently supported by postmortem findings, and in 1 patient (case G (55)) Hodgkin's disease was undoubtedly also present and probably anteceded the fungous infection. In this patient, the lesions of histoplasmosis and those of Hodgkin's disease were intermingled in the bone marrow The occurrence of 4 cases of malignant lymphoma among approximately 90 cases of histoplasmosis can only with difficulty be attributed to chance alone.

Through the courtesy of Dr. J K. Kuzma, the author was able to study a case which is of particular interest in this connection Shortly before death, this patient had a red blood cell count of 850,-000, and a white cell count of 1,400, with lymphocytes predominating At autopsy, the liver showed a leukemialike infiltration of lymphocytes in the portal areas (Fig 8) The bone marrow, however, was extensively replaced by mononuclear cells loaded with Histoplasma It seemed possible to explain the entire picture on the basis of histoplasmosis

In the case reported by Williams and Cromartie (80), which was believed to be a combination of leukemia and histoplasmosis, the blood picture showed 3,820,000 erythrocytes and 12,100 white blood cells, of which 29 per cent were polymorphonuclears and 71 per cent were adult lymphocytes The liver weighed 2,900 Gm, the spleen 400 Gm, and a mass of matted mesenteric lymph nodes 620 Gm Microscopically, the liver, spleen, and bone marrow showed "evidence of chronic lymphatic leukemia" A white cell count of only 12,100 and a spleen weighing only 400 Gm are not usual in untreated chronic lymphatic leukemia

It is well known that leukemialike infiltration of the liver, spleen, and kidney may occur when the bone marrow is extensively destroyed by tumor invasion, as well as in certain prolonged infectious processes Although the infiltrating cells are usually myeloid in character, they may be nongranular, and may be indistinguishable from lymphocytes (59) For this reason, it may be difficult to

Fig 6 Low power appearance of a greatly enlarged lymph node, showing many pale cells of reticuloendothelial origin loaded with Histoplasma organisms

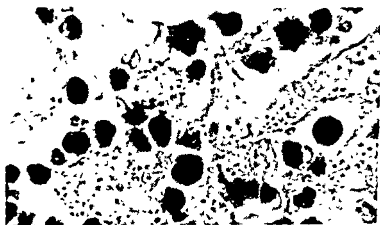


Fig 7 High power appearance of lymph node, showing Histoplasma organisms in swollen reticular cells

findings were those which ordinarily lead to a diagnosis of bacterial endocarditis; and except for the presence of the Histoplasma organisms at the base of the vegetations and the occurrence of granulomatous lesions in the viscera, the pathologic picture too was identical with that of bacterial endocarditis. The case reported by Broders and co-workers (8) was diagnosed during life as histoplasmosis only because repeatedly negative blood cultures led to an exploratory laparotomy with biopsy of the liver. (It is possible that blood cultures might have been found positive for Histoplasma if they had been observed for a period of 15 days.)

The case reported by Beamer, Reinhardt, and Goodof (6) had been diagnosed as bacterial endocarditis, even after postmortem examination. The correct diagnosis was finally made by reviewing the microscopic sections six years after the autopsy. The two last-mentioned patients (6,8) showed chills and fever, headache, weight loss, and increasing weakness over a period of 7 to 8 months. In one case, cardiac enlargement was seen on the roentgenogram. Both patients had systolic murmurs early in the course of the disease. The electrocardiogram in one case showed only right axis deviation. The vegetations were large and friable in both cases, and infarction of the spleen was noted. It is perhaps interesting that in both cases there was evidence suggestive of previous cardiac damage, old rheumatic fever in one case (8) and "syphilitic aortic valvulitis" in the other (6).

In the remaining two cases (63,57), clinical evidence of endocarditis was not present, and it seems likely that the cardiac involvement was a terminal event. One of these cases (57) was initially of the oral type. From a study of the first 2 cases, however, it is clear that histoplasmosis must be considered in the differential diagnosis of cases showing clinical evidence of subacute endocarditis, particularly when repeated blood cultures fail to show the usual bacterial etiology.

Beamer *et al.* (6) discuss the etiologic role in endocarditis of the higher bacteria and fungi in general.

Pulmonary Type. In several cases, the clinical picture has suggested primary disease of the lungs, and roentgenologic evidence (localized shadows and cavities) has often led to a diagnosis of pulmonary tuberculosis. Pulmonary involvement is frequently present in the generalized type of Histoplasma infection (Fig. 9).

distinguish between the leukemoid reactions resulting from histoplasmosis and true leukemia.



Fig 8. Portal lymphocytic infiltration in a case of histoplasmosis with massive involvement of the bone marrow The picture closely resembles that of lymphatic leukemia.

The relationship between histoplasmosis and malignant lymphoma requires further study Parsons and Zarafonetis (57) suggested that in their case the possible presence of Hodgkin's disease may have lowered the resistance to histoplasmosis A similar explanation may account for the simultaneous occurrence of lymphatic leukemia and histoplasmosis, or conceivably leukemia may be activated by histoplasmosis.

Cardiac Type. Verrucous endocarditis, involving the cardiac valves, has been found in 4 cases (6,8,57,63,81) All 4 patients were men, 3 in the fifth and sixth decades of life The aortic, mitral, and tricuspid valves were involved in various combinations In 2 of these cases (6,8), the clinical course and laboratory and physical

evidence of generalized infection, even on postmortem examination This suggests the possibility that the respiratory tract may have been the portal of entry. It is possible also that an area of tuberculous infection may serve as a point of entry, as is believed to be true in Torula infection



Fig 10 Flask-shaped ulcer in the colon of a patient with intestinal histoplasmosis

At present, there is reason for believing that Histoplasma by itself can produce lesions which are roentgenologically indistinguishable from those of tuberculosis The findings in one of Meleney's cases suggest that cavitation may occur in uncomplicated Histoplasma infection Whether such lesions may become chronic and eventually heal, leaving residual calcification in the lungs (page 224), is at present an unanswered question

Intestinal Type. Involvement of the gastrointestinal tract is common, and ulcers (Fig 10) or nodules have been found in the pylorus of the stomach, in the jejunum, ileum, caecum, large intes-

The diagnosis of pulmonary histoplasmosis is somewhat complicated by the fact that tuberculosis may be present concurrently.

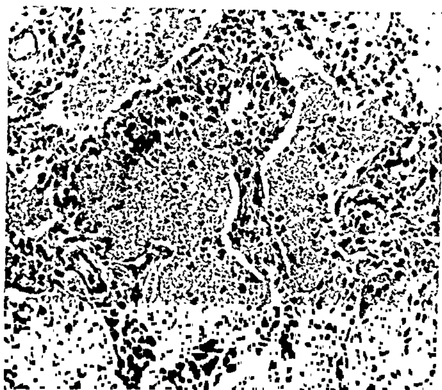


Fig 9 Interstitial pneumonitis in systemic histoplasmosis Pale phagocytic cells, laden with organisms, crowd the alveolar walls and lie free within the alveoli

In 2 cases reported by Meleney (45) there was an associated tuberculous infection In view of the similarity of the lesions produced by the two agents differentiation may be possible only when the etiologic agent is found in the lesions In some cases, tubercle bacilli and Histoplasma organisms have been found side by side

There appear to be no important clinical differences between cases of histoplasmosis associated with tuberculosis and those which are not In both groups, irregular fever, night sweats, cough, emaciation, and chest pain have been the outstanding symptoms

In several cases of pulmonary histoplasmosis, there has been no

chest showed extensive lung involvement, probably by Histoplasma. The patient died shortly after the operation, and permission for autopsy could not be obtained



Fig 11 Caseous necrosis in the adrenal gland There is complete necrosis in the upper right corner while the tissue in the lower left corner is essentially normal

Adrenal Involvement. Caseous necrosis of the adrenals is a very common type of lesion in histoplasmosis (Fig 11), and it is probable that the hypotension recorded in 3 cases was caused by the adrenal lesions which were found at autopsy In 1 case, summarized by Parsons and Zarafonetis (57), splenomegaly and hepatomegaly were present, and toward the end of the patient's illness there was evidence of adrenal insufficiency Death occurred in spite of treatment with adrenal extract At autopsy, necrosis of both adrenals was found, due to histoplasmosis, no Histoplasma organisms, however, were found in any other organ

tine, and rectum Diarrhea has been present in at least 12 cases In some cases, ulcerative enteritis is probably a terminal event, occurring late in the course of systemic infection.

In at least 3 cases (32), however, diarrhea appeared early and was so persistent and prominent that the problem was one of finding an etiologic agent for ulcerative enteritis One case (32) was mistaken clinically for amebic dysentery. It is probable that macrophages containing Histoplasma organisms were seen in stools and were believed to be amebas with ingested erythrocytes.

The signs of this type of histoplasmosis are emaciation, anorexia, fever, asthenia, and diarrhea, with distention and slight tenderness of the abdomen Vomiting may or may not occur. The stools are frequent, whitish, and liquid and may contain gross blood Alternating diarrhea and constipation were noted in 1 case. The cases with early or late intestinal involvement that had occurred up to 1942 were reviewed in that year by Henderson, Pinkerton, and Moore (32), who followed Crumrine and Kessel (15) in suggesting the importance of examining the stool for fungi in cases of ulcerative enteritis of obscure etiology

De Monbreun (23) was able to infect dogs by feeding them with culture of Histoplasma In 1 case of spontaneous infection in a dog, he believed that histologic evidence suggested the gastrointestinal route of infection Humphrey (35) also believed the gastrointestinal tract to be the portal of entry in his second case Involvement of the mesenteric lymph nodes is almost constant in fatal cases of histoplasmosis In those cases in which diarrhea occurs early and is persistent, the evidence for primary infection by way of the intestinal tract appears to be good In other cases, it may be terminal, and is then comparable to the ulcerative enteritis in fatal pulmonary tuberculosis

Joint Involvement. The case reported by Key and Large (37) is unique The presenting clinical feature in this patient was marked inflammation and extensive destruction of the knee joint The case resembled tuberculosis of the knee joint, but this was ruled out by guinea pig inoculation Chronic pyogenic infection appeared to be the most likely diagnosis An amputation was performed, in the synovial membrane and adjacent bone tissue large numbers of Histoplasma capsulatum organisms were found Roentgenograms of the

chest showed extensive lung involvement, probably by Histoplasma. The patient died shortly after the operation, and permission for autopsy could not be obtained.

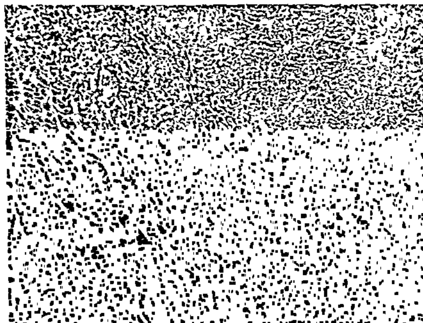


Fig 11 Caseous necrosis in the adrenal gland There is complete necrosis in the upper right corner while the tissue in the lower left corner is essentially normal

Adrenal Involvement. Caseous necrosis of the adrenals is a very common type of lesion in histoplasmosis (Fig 11), and it is probable that the hypotension recorded in 3 cases was caused by the adrenal lesions which were found at autopsy. In 1 case, summarized by Parsons and Zarafonetis (57), splenomegaly and hepatomegaly were present, and toward the end of the patient's illness there was evidence of adrenal insufficiency Death occurred in spite of treatment with adrenal extract At autopsy, necrosis of both adrenals was found, due to histoplasmosis, no Histoplasma organisms, however, were found in any other organ.

INFANTILE HISTOPLASMOSIS

Its occurrence in infancy and childhood has been reviewed particularly by Anderson, Michelson, and Dunn (3), by Rhodes, Conant, and Glesne (65), and by Iams, Tenen, and Flanagan (36) The last mentioned suggest the possibility of intrauterine infection in cases occurring during the first 3 months of life. Proof of this concept (which is established in cases of Toxoplasma infection) has not been found The peak incidence for the disease is during the first year of life.

The majority of the cases of infantile histoplasmosis are of the systemic or generalized type (2,25,67), with enlargement of liver and spleen, fever, and failure to gain weight or loss of weight. Diarrhea, vomiting, evidence of upper respiratory infection, and otitis media have been noted. Eventually, consolidation of the lungs has occurred in nearly all of the cases, and ulceration of the intestinal tract has been especially common In the case described by Anderson *et al*, a clinical diagnosis of aleukemic leukemia was made.

RELATION TO PULMONARY CALCIFICATION

It has already been stated that histoplasmosis, as far as definite knowledge goes, is almost invariably fatal However, it is obviously unwise to draw conclusions regarding the prognosis of a disease so long as the diagnosis is made only at autopsy or by biopsy taken from patients with systemic infection or with localized nodular or ulcerative lesions

It is now known that coccidiodomycosis, which prior to 1930 was thought to be invariably fatal, usually heals, with no more serious permanent sequelae than residual pulmonary calcification (4) It should be emphasized that most of the healed cases of coccidiodomycosis had no clinical manifestations, and none of them showed the chronic granulomas described in all the clinical cases prior to 1930 and in the fatal cases since then It seems possible that the same may be true of histoplasmosis In recent years, several investigators have become interested in this possibility, and the results of their studies, so far as they go, tend to support such a theory

For many years there has been speculation concerning the etiology of the so-called disseminated pulmonary calcification—a condition

in which multiple calcareous deposits, from "buckshot" size to a centimeter or more in diameter, are scattered throughout the lungs and hilar region The older theory that these lesions all represent healed miliary or conglomerate tubercles is not well supported by immunologic evidence. Multiple calcification of the spleen and liver, which also has been ascribed to healed tuberculosis, is another lesion which has not been satisfactorily explained

Statistical study has shown that there is a definite concentration of the cases of disseminated pulmonary calcification in the central region of the United States—the very region in which histoplasmosis has most often been recognized (54). Furthermore, the great majority of cases with this roentgenologic feature have a negative reaction to the tuberculin test and a positive reaction to the histoplasmin test.

The specificity of the histoplasmin test has been questioned Emmons and associates (26) found that sensitivity to haplosporangin was absent in experimental animals infected with Histoplasma capsulatum, Blastomyces dermatitidis, and Coccidioides immitis. These same workers, however, found that 34 of 136 hospital patients selected at random gave positive reactions to both histoplasmin and blastomycin Christie and Peterson (11) found that humans with pulmonary calcification who were histoplasmin positive were haplosporangin* negative

There can be no doubt that there is a striking relationship between occurrence of sensitivity to histoplasmin and development of pulmonary calcification (28,33,43,53), but in no instance has an active pulmonary lesion, proved to be due to histoplasmosis, been shown to heal Evidence that this does occur might be obtained by culture studies of the sputum If pulmonary histoplasmosis does heal, the resulting lesion would undoubtedly calcify (Calcification in a lymph node with active Histoplasma infection may be seen in Figure 12)

Emmons *et al* (26) state that they will report later the case of a child with pulmonary calcification and in whom Histoplasma infection of the hilar lymph nodes was apparently only a concomitant

*Haplosporangin is an antigen derived from Haplosporangium parvum, a fungus which causes granulomatous lesions in the lungs of certain rodents This organism has not been shown to infect man, it is of interest only in respect to the possible occurrence of cross reactions among fungous antigens.

infection. On the whole, it seems best to reserve judgment as to the role of Histoplasma capsulatum in the production of pulmonary calcification without clinical symptoms. Further clinical and pathologic studies of this problem are needed.

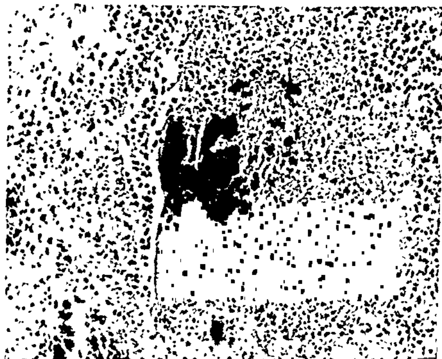


Fig 12 Appearance of lymph node with histoplasmosis, necrosis and calcium deposits are in evidence, and many organisms are still present

Diagnosis

Awareness of the various clinical forms which histoplasmosis may assume is helpful when making a diagnosis Histoplasmosis is differentiated from a large number of more common conditions which clinically resemble it by a variety of methods, all of which involve demonstration that the etiologic agent, Histoplasma capsulatum, is present In well-stained preparations, morphologic criteria are adequate for a definite diagnosis by one familiar with the appearance of the organism Culture features are even more definite In approxi-

mately one-third of the reported cases, diagnosis was established while the patient was still alive In the majority of the remaining cases, which were diagnosed only from postmortem study, a diagnosis during life could undoubtedly have been made, had the condition been suspected. Diagnosis by biopsy has been the most successful of all diagnostic methods during life

Biopsy. Material removed by biopsy should be cultured and injected into animals, in addition to being studied histologically. Enlarged superficial lymph nodes, and cutaneous, mucocutaneous, or naso-oral lesions are most readily examined. In one case, biopsy of the liver at the time of an exploratory laparotomy led to the correct diagnosis Aspiration biopsy of the liver, although apparently an innocuous procedure, cannot often give a positive result when other methods are negative, and should be avoided in cases with purpuric tendencies.

While fungi are usually present in large numbers, in rare instances they have been found only after a long search. Tuberclelike lesions caused by Histoplasma, but without visible organisms, are often found in various organs at autopsy.

Sternal Marrow Puncture. In 6 out of 10 cases in which this procedure was carried out, the presence of Histoplasma has been demonstrated The organisms are readily identified within phagocytic cells of reticuloendothelial origin, and at times in neutrophils. Their appearance is shown in Figures 13 and 14.

Blood Smears. Histoplasma may be identified in blood smears in cases of the generalized type The organisms, which are present in monocytes and polymorphonuclear neutrophils, appear much as they do in sternal marrow smears A diagnosis has been established by this method in 5 cases, but obviously a negative result has little significance Although the organisms may be seen in ordinary film preparations, thick films, such as those used for the diagnosis of malaria, probably increase the chances of success Giemsa's and Wright's stains are adequate for their recognition.

Culture This method, by analogy with other organisms, is probably a more sensitive indicator than direct observation. Material from stools and sputum may be cultured, as well as biopsy material of various types. The organism grows less rapidly on Sabouraud's agar than on blood agar, but the former is valuable when working with contaminated material In any case, cultures should be kept

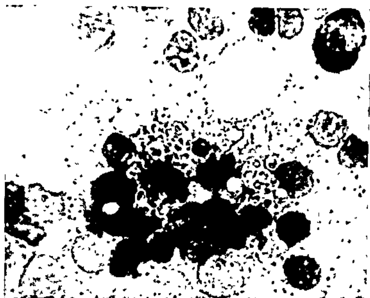


Fig 13 Film preparation from material obtained by sternal marrow puncture, stained with Giemsa's stain Two cells containing organisms are shown

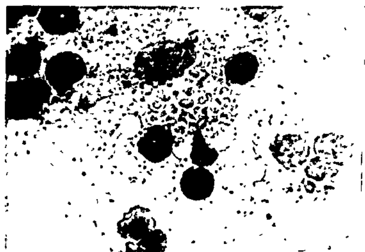


Fig 14 Another infected cell from the same preparation as that used for Figure 13

for at least 15 days before being discarded Successful cultures have been obtained as follows: 18 from biopsy or autopsy material, 9 from peripheral blood, and 1 from sternal marrow

The use of the egg (48) as a culture medium for initial isolation might be found valuable when sterile material is available. The sensitivity of this method has not been investigated

Animal Inoculation This method seems likely to prove valuable because it offers a possibility of using it for the isolation of organisms from contaminated material. Of the various animals which are susceptible, young mice are probably most suitable for diagnostic purposes (57) Injection may be made intravenously, intraperitoneally, or subcutaneously The latter route is most likely to filter out contaminating organisms.

Stools. Diagnosis has apparently not been made by stool examination, although Histoplasma organisms were probably seen in the case reported by Henderson *et al* (32). In cases with ulcerative enteritis, routine direct examination and culture of the stools for fungi would probably lead to a diagnosis in rare cases.

Sputum Diagnosis from the sputum has not been reported, and probably has not often been attempted Methods for collecting, preparing, examining, and culturing the sputum in cases of suspected fungous infection have been discussed by Kurung (38). This paper also contains good illustrations of the cultural and morphologic features on which differential diagnosis is based. If a mild form of pulmonary histoplasmosis, with recovery and residual calcification of the lung fields, is to be established as a definite entity, it would seem that culture studies by such methods as those described by Kurung might play an important role

Cutaneous Test The methods for preparing the antigen have been described by Zarafonetis and Lindberg (82) and by Van Pernis, Benson, and Hollinger (74) The former workers used (*1*) a sterile filtrate (Berkefeld N filter) of a 7 weeks' growth of the mycelial form of the organism grown in Williams synthetic medium, or (*2*) a suspension of about one billion yeastlike organisms per cubic centimeter, grown on blood agar slants and suspended in physiologic saline containing 0.5 per cent of formalin The latter workers used a filtrate of a dextrose broth culture, or the acetone-precipitated substance of a broth filtrate obtained by treating it with 3 volumes of acetone and redissolving the precipitate in saline

The antigen is injected intradermally, and the occurrence of an erythematous wheal is interpreted as a positive reaction.

If these tests prove to be reliable, they should be of great importance in finding cases of active hisplasmosis and in proving past infection in recovered cases with pulmonary calcification or other residual lesions The possible occurrence of cross reactions with other fungi needs further study.

DIFFERENTIAL DIAGNOSIS

The conditions to be considered in the differential diagnosis of histoplasmosis are for the most part more common than the latter disease. For this reason it is probably more accurate to say that histoplasmosis should be considered in the differential diagnosis of these diseases, which are of relatively common occurrence.

The cutaneous form of the disease may simulate other mycotic infections, such as blastomycosis and actinomycosis, *Dehli boil*, leukemia cutis, tuberculosis, leishmaniasis, certain types of dermatitis exfoliativa, and in infants, impetigo Ulcerative lesions on the lips resemble those of noma The lesions on the penis may be mistaken for those of syphilis Nasal lesions may resemble papillomas When it occurs in the mouth, particularly on the tongue, and also in the larynx, an incorrect diagnosis of carcinoma has often been made *Blastomycosis and tuberculosis must be ruled out, the latter* especially when it occurs in the larynx The otitic form may clinically resemble otitis media caused by more common incitants

The generalized form of the disease must be distinguished from other subacute and chronic conditions associated with fever, splenomegaly, hepatomegaly, enlargement of lymph nodes, anemia, leukopenia, weight loss, diarrhea, and vomiting, these are notably miliary *tuberculosis, kala azar, typhoid fever, brucellosis, malaria, and* amebiasis The blood picture, splenomegaly, and enlarged lymph nodes have led to the diagnosis of leukemia, aleukemic leukemia, lymphosarcoma, and Hodgkin's disease, and might be mistaken for Banti's disease or portal cirrhosis of the liver

Histoplasmosis with diarrhea and ulcerative enteritis must be differentiated from amebic dysentery, bacillary dysentery, tubercu*lous enteritis, ulcerative colitis, regional enteritis and other types of* enteritis The cardiac form of the disease can be distinguished from

bacterial endocarditis only by finding the causative organism Adrenal involvement may simulate Addison's disease. Pulmonary infection, with the accompanying roentenlogic features, is most suggestive of tuberculosis, which may in fact coexist.

Diagnosis of the various types of histoplasmosis may be suggested by a positive cutaneous test, but can be made definitely only by demonstrating the presence of Histoplasma capsulatum

Prognosis

Disregarding the possibility that certain types of pulmonary calcification may represent healed histoplasmosis, the disease hitherto clinically recognized is nearly always fatal The duration of life, however, varies widely, ranging from a few weeks to 15 years The majority of cases live less than a year The best prognosis can be given for those patients who have chronic ulcerative lesions on the skin, mucocutaneous surfaces, or naso-oral cavity, and who show no evidence of systemic involvement. Parsons and Zarafonetis (57) stated that 4 cases of this type lived for 6, 5, 2, and 2 years, respectively, after the diagnosis was established One patient (case H) whom they report had recurrent ulcers of the tongue, which were treated with radiotherapy, 5 years later this patient was not only alive but showed no manifestations of the disease. Of the fatal cases, 4 lived with the infection for 4, 8, 10 and 16 years, respectively When evidence of systemic infection appears, death usually occurs in a few weeks or months All infants under 1 year of age with the disease have died less than 6 months after the diagnosis was made

Treatment

A wide variety of therapeutic agents have been tested, none of which has led to definite success One case, mentioned above, is well after radiotherapy, but in several other cases, including both localized and generalized types of the disease, such treatment has been ineffective, or possibly even deleterious

Surgery would appear to have little to offer, but might well be tried when the disease appears to be localized and the lesions accessible The development of active or passive immunity has not been extensively studied, but autogenous vaccines were not successful in

one case. The immunologic studies of Scheff (66) have been referred to under "Etiology"

A wide variety of chemotherapeutic agents have given negative or equivocal results. These include the iodides, arsenicals, antimony preparations, pentnucleotide, sulfanilamide, sulfathiazole, sulfapyridine, atebrine, and quinine. Neostam (stibamine glucoside), an antimony preparation, was believed to have been beneficial in one case, but the results in other cases are difficult to evaluate (57).

The chemotherapy of histoplasmosis should be carefully studied in experimental animals under controlled conditions. The only reported work of this type which the author was able to find is that by Levy (41), who obtained negative results with sodium iodide, neostam, fuadin, sulfanilamide, proflavine, thymol, an organic iodide identified by the symbol "B9," and sodium propionate

References

1 Agress, H, and Gray, S H Histoplasmosis and reticulo-endothelial hyperplasia Am J Dis Child *67*, 573, 1939

2 Amolsch, A L, and Wax, J N Histoplasmosis in infancy. Report of a case Am J Path *15*, 477, 1939

3 Anderson, W A D, Michelson, I D, and Dunn, T M Histoplasmosis in infancy Report of a case Am J Clin Path *11*, 344, 1941

4 Aronson, J D, Saylor, R M, and Parr, E I · Relationship of coccidiomycosis to calcified pulmonary nodules Arch Path *34*, 31, 1942

5 Balina, P L, Negroni, P, Bosq, P, and Herrara, J A Histoplasmosis de Darling Primer caso sud americano Rev argent dermatosif *25*, 491, 1941

6 Beamer, P R, Reinhardt, E H, and Goodof, I I Vegetative endocarditis caused by higher bacteria and fungi, review of previous cases and report of 2 cases with autopsies Am Heart J *29*, 99, 1945

7 Boltjes, B Histoplasmosis Report of a case with brief review of the literature J Kansas M Soc *44*, 226, 1943

8 Broders, A C, Dochat, G R, Herrell, W E, and Vaughn, L D Histoplasmosis producing vegetative endocarditis J A M A *122*, 489, 1943

9 Brown, A E, Havens, F Z, and Magath, T B Histoplasmosis Report of case Proc Staff Meet, Mayo Clin *15*, 812, 1940

10 Callahan, W P, Jr Spontaneous histoplasmosis occurring in a dog Am J Trop Med *24*, 263, 1944

11 Christie, A, and Peterson, J C Pulmonary calcification and sensitivity to histoplasmin, tuberculin, and haplosporangin J A M A *131*, 658, 1946

12 Ciferri, R, Redaelli, P, and Visocchi, V The Histoplasmaceae Family, synthetic review Mycopathologia *1*, 101 1938

13. Clemens, H H, and Barnes, M L Histoplasmosis of Darling Report of a case South M J *33*, 11, 1940

14 Conant, N F A cultural study of the life-cycle of Histoplasma capsulatum Darling 1906, J Bact *41*, 563, 1941.
15 Crumrine, R M, and Kessel, J F Histoplasmosis (Darling) without splenomegaly Am J Trop Med *11*, 435, 1931
16 Darling, S. T ; A protozoon general infection producing pseudotubercles in the lungs and focal necroses in the liver, spleen and lymph nodes J. A M A *46*, 1283, 1906
17. Darling, S. T · Histoplasmosis· A fatal infectious disease resembling kala-azar found among natives of tropical America Arch Int Med *2*, 107, 1908
18 Darling, S T The morphology of the parasite (Histoplasma capsulatum) and the lesions of histoplasmosis A fatal disease of tropical america J Exper Med & Hyg *11*, 515, 1909
19 da Rocha-Lima, H Histoplasmosis und epizootische Lymphangitis Beihefte z Arch f Schiffs- u Tropen-Hyg *16*, 79, 1912.
20 da Rocha-Lima, H Beitrag zur Kenntnis der Blastomykosen Lymphangitis epizootica und Histoplasmosis Centralbl f. Bakteriol I Abt *67*, 233, 1913
21 Dean, L W, Jr Histoplasmosis of the larynx Report of a case Arch Otolaryng *36*, 390, 1942
22 De Monbreun, W A The cultivation and cultural characteristics of Darling's Histoplasma capsulatum Am J Trop Med *14*, 93, 1934
23 De Monbreun, W A, and Anderson, K The dog as a natural host for Histoplasma capsulatum Report of a case of histoplasmosis in this animal Am J Trop Med *19*, 565, 1939
24 Derry, D C L, Card, W I, Wilson, R, and Duncan, J T : Histoplasmosis of Darling Report of a case, mycologic note Lancet *1*, 224, 1942
25 Dodd, K, and Tompkins, E H A case of histoplasmosis of Darling in an infant Am J Trop Med *14*, 127, 1934
26 Emmons, C W, Olson, B T, and Eldridge, W W Role of fungi in pulmonary disease, cross reactions of histoplasmin Pub Health Rep *60*, 1383, 1945
27 Feder, J M, and Tribble, E Histoplasmosis Brief review of available literature South Med & Surg *106*, 20, 1944
28 Furcolow, M L, High, R H, and Allen, M F Some aspects of sensitivity to histoplasmin and tuberculin Pub Health Rep *61*, 1132, 1946
29 Gunter, W A, and Lafferty, C Histoplasmosis of Darling Report of a case J M A Alabama *9*, 337, 1940
30 Hansmann, G H, and Schenken, J R New disease caused by a yeast-like organism Science *77*, 460, 1933
31 Hansmann, G H, and Schenken, J R A unique infection in man caused by a new yeast-like organism, a pathogenic member of the genus sepedonium Am J Path *10*, 731, 1934
32 Henderson R G, Pinkerton, H, and Moore, L T Histoplasma capsulatum as a cause of chronic ulcerative enteritis J A M A *118*, 885, 1942
33 High, R H, Zeverling, H B, and Furcolow, M L Disseminated pulmonary calcification A report of 113 cases Pub Health Rep *62*, 20, 1947

34. Howell, A , Jr Studies on Histoplasma capsulatum and similar form species I Morphology and development Mycologia 31, 191, 1939
35 Humphrey, A A Reticuloendothelial cytomycosis (histoplasmosis of Darling) Arch Int Med 65, 902, 1940
36 Iams, A M , Tenen, M M , and Flanagan, H F · Histoplasmosis in children; review of literature with report of a case. Am J. Dis Child 70, 229, 1945
37 Key, J A , and Large, A M Histoplasmosis of the knee J Bone & Joint Surg 24, 281, 1942
38 Kurung, J M The isolation and identification of pathogenic fungi from sputum Am Rev Tuberc 46, 367, 1942
39 Kuzma, J K , and Schuster, M Histoplasmosis—reticulo-endothelial cytomycosis, report of a case from Wisconsin Wisconsin M. J. 45, 591, 1946
40 Levine, N D, Dunlap, G L, and Graham, R · An intracellular parasite encountered in the ferret. Cornell Vet 28, 249, 1938
41. Levy, B M . Chemotherapy of experimental histoplasmosis in white mice Am J Trop Med 25, 241, 1945
42 Levy, B M Oral manifestations of histoplasmosis J Am Dent A 32, 215, 1945
43 Mantz, H L Histoplasmin sensitivity and pulmonary calcification, review Journal-Lancet 66, 100, 1946
44 Meleney, H E Histoplasmosis (reticulo-endothelial cytomycosis) A review Am J Trop Med 20, 603, 1940
45 Meleney, H E Pulmonary histoplasmosis Report of two cases Am Rev Tuberc 44, 240, 1941
46 Moore, M Posadasia pyriformis and P capsulata, two causative organisms of Darling's histoplasmosis in the United States Ann Missouri Botan Garden 21, 347, 1934
47 Moore, M A morphological and physiological study of two species of Posadasia Ann Missouri Botan Garden 22, 335, 1935
48 Moore, M Histoplasma capsulatum Its cultivation on the chorio-allantoic membrane of the developing chick and resultant lesions Am J Trop Med 21, 627, 1941
49 Moore, M , and Jorstad, L H Histoplasmosis and its importance to otorhinolaryngologists, review with report of a new case Ann Otol Rhin and Laryng 52, 779, 1943
50 Muller N Histoplasmosis in Oost Java, Geneesk Tijdschr v Nederl-Indie 72, 889, 1932
51 Nègre, L , and Bridre, J Un cas de lymphangite épizootique chez l'homme Traitement et guérison par le "606" Bull Soc path exot 4, 384, 1911
52 Negroni, P Estudio micrologico del primer caso argentino de histoplasmosis Fol biol p 390, 1938
53 Palmer, C E Nontuberculous pulmonary calcification and sensitivity to histoplasmin Pub Health Rep 61, 475, 1945
54 Palmer, C E Geographic differences in sensitivity to histoplasmin among student nurses Pub Health Rep 61, 475, 1946

55 Parsons, R J.: Experimental histoplasmosis in mice, Arch. Path. *34,* 229, 1942
56 Parsons, R J., and Everett, M. Histoplasmosis in a dog: Report of a case To be published
57 Parsons, R J, and Zarafonetis, C. J. D: Histoplasmosis in man; report of 7 cases and review of 71 cases Arch Int. Med *75,* 1, 1945
58 Phelps, B M, and Mallory, F B. Toxic cirrhosis and primary liver cell carcinoma complicated by histoplasmosis of the lung In Fifteenth Annual Report of the Medical Department of the United Fruit Company, p. 115 New York, United Fruit Company, 1926
59 Pinkerton, H · Aleukemic leukemia and atypical leukemoid conditions Arch Path 7, 567, 1929
60 Pinkerton, H, and Weinman, D · Toxoplasma infection in man Arch Path *30,* 374, 1940
61 Reid, J D, Scherer, J. H, and Irving, H: *Systemic histoplasmosis in the* United States Science *91,* 264, 1940
62 Reid, J D., Scherer, J H, Herbut, P A, and Irving, H.: Sytemic histoplasmosis diagnosed before death and produced experimentally in guinea pigs J Lab & Clin Med *27,* 419, 1942
63 Riehl, G, Jr Durch pathogene Sprosspilze bedingte Granulome Arch f Dermat u Syph *145,* 392, 1925.
64 Redaelli, P, and Ciferri, R Études sur l'Histoplasma capsulatum Darling 1 Réproduction expérimentale de l'histoplasmoses et définition de la maladie comme réticolo-histocytose parasitaire atteignant divers systèmes de l'organisme Boll Sez ital Soc internaz, microb *6,* 193, 1934
65 Rhodes, P H, Conant, N F, and Glesne, L R. B: Histoplasmosis Report of case in an infant 3 months of age J Pediat *18,* 235, 1941
66 Scheff, G J Biochemical and immunologic properties of Histoplasma capsulatum Yale J Biol & Med *18,* 41, 1945
67 Schlumberger, H G, and Service, A C A case of histoplasmosis in an infant with autopsy Am J M Sc *207,* 230, 1944
68 Shaffer, F J, Shaul, J F, and Mitchell, R H: Histoplasmosis of Darling Fourth Case to be Reported in United States J A M A *115,* 484, 1939
69 Simson, F W, and Barnetson, J Case of histoplasmosis J Path & Bact *54,* 299, 1942
70 Strong, R P A study of some tropical ulcerations of the skin with particular reference to their etiology Philippine J Sc *1,* 91, 1906
71 Tager, M, and Liebow, A A · Observations on histoplasmosis Induced infection in the mouse Yale J Biol & Med *14,* 469, 1942
72 Thuringer, J M Histoplasmosis · Report of its occurrence in a dog Arch Path *37,* 140, 1944
73 Tomlinson, W J, and Grocott, R G. Canine histoplasmosis. Pathologic study of three reported cases and first case found in Panama Canal Zone Am J Clin Path *15,* 501, 1945
74 Van Pernis, P A, Benson, M E, and Hollinger, P H · Specific cutaneous reactions with histoplasmosis Preliminary report of another case. J A M A *117,* 436, 1941.

75. Van Pernis, P. A., Benson, E., and Hollinger, P. H.: Laryngeal and systemic histoplasmosis (Darling). Ann. Int. Med. *18*, 384, 1943
76. Villela, E., and Madureira Para: Histoplasmose em criança no estado de Minas Gerais. Rev. brasil. de biol. *1*, 449, 1941.
77. Wade, W. W.: A case of systemic mycosis due to a fungus of uncertain classification. Report to Culion Medical Society Culion, Philippine Islands, 1926
78. Watson, C. J., and Riley, W. A.: A case of Darling's histoplasmosis originating in Minnesota. Arch. Path. *1*, 662, 1926
79. Watson, C. J.: The pathology of histoplasmosis (Darling) with special reference to the origin of the phagocytic cells. Folia haemat. *37*, 70, 1928
80. Williams, R. H., and Cromartie, W. J.: Histoplasmosis: Report of a case. Ann. Int. Med. *13*, 2166, 1940.
81. Wood, W. B., Jr., and Moore, R. A.: Case reports of Barnes Hospital. Clinical and postmortem records used in weekly clinico-pathologic conferences at Barnes Hospital, St. Louis (Case 22), J. Missouri State M. A. *40*, 251, 1943.
82. Zarafonetis, C. J. D., and Lindberg, R. B.: Histoplasmosis of Darling; observations on antigenic properties of causative agent; preliminary report. *Univ. Hosp. Bull., Ann Arbor 7, 47, 1941.*

Treatment of Hyperthyroidism with Antithyroid Compounds

E. B. Astwood

Joseph H. Pratt Diagnostic Hospital and Tufts College Medical School, Boston, Massachusetts

Introduction

During the past few years there have been many publications on newer methods of treatment for hyperthyroidism. These have dealt with the use of antithyroid compounds for preparing patients for thyroidectomy, the use of these compounds as a definitive treatment, and the administration of radioactive iodine for internal irradiation of the thyroid gland Various combinations of these newer therapeutic measures have also been suggested.

It is not the object of this review to assess the relative merits of the several proposed methods of treatment but only to summarize the available information on the use of antithyroid compounds as the chief, if not the sole, form of treatment. Even with the subject thus restricted, the medical literature is already too extensive for complete coverage, nor does it seem practical to document all of the conclusions reached by citing the large number of reports pertaining thereto.

If antithyroid compounds are to be used for the treatment of hyperthyroidism, it is of importance that as much as possible be learned of their mechanism of action. It would also be useful to know the optimal dosage, the proper duration of treatment, the probability of a lasting remission, and the effects of antithyroid therapy on the various secondary manifestations of hyperthyroidism, as well as the complications

Mechanism of Action

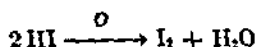
Despite extensive studies, the ultimate site of action of antithyroid compounds is as yet unknown It is generally agreed that antithy-

roid compounds act in such a way as to inhibit thyroid hormone synthesis, but the normal synthetic process leading to the formation of thyroid hormone within the thyroid gland is not completely understood Certain features of this process have been clearly established, and an approach has been made to a definition of the mode of action of antithyroid compounds.

Iodine from the diet reaches the thyroid gland via the circulation and is absorbed in the form of iodide ion. Recent studies by Schachner, Franklin, and Chaikoff (50) and VanderLaan and VanderLaan (58) have established that there is a selective mechanism within thyroid tissue for the accumulation of iodide ion from the blood. The VanderLaans have shown that the normal resting thyroid of experimental animals maintains a concentration of iodide within itself some 25 times that of the circulating blood Under conditions of hyperplasia, the thyroid gland apparently develops a capacity for holding a much higher concentration than this, and under certain circumstances the iodide concentration within the thyroid is 250 times that of the blood This selective iodide-collecting mechanism is specifically inhibited by thiocyanate ion (57,58,67). No other substance has yet been found to exert an effect similar to thiocyanate, and apparently antithyroid compounds in no way interfere with the accumulation of iodide ion within the thyroid gland. Thus, the site of action of thiocyanate has been very specifically localized, but until it is known how the thyroid manages to concentrate iodide ion the mechanism of action cannot be defined more closely.

Antithyroid compounds act in the next step of thyroid hormone synthesis. This step involves the oxidation of iodide ion to what would correspond to free iodine (Fig. 1). Presumably, the iodine promptly iodinates tyrosyl radicals of thyroid protein It is unknown whether iodine ever exists in the free form within thyroid tissue, and it may be that the oxidation of iodide and the coupling of iodine to the benzene ring occur simultaneously. In any event, it would appear that compounds of the thiouracil type act by preventing the oxidation of iodide ion or by the reduction of iodine as fast as it is formed (34) (Fig 2). In effect, therefore, thiouracil-like compounds prevent the organic binding of iodine

The final step in thyroid hormone synthesis presumably involves the oxidative coupling of two diiodotyrosyl radicals to form thy-



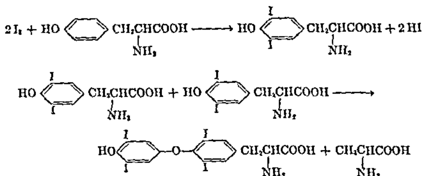


Figure 1

roxyl groups. Little is known about the mechanism of this step, but it has been shown to occur *in vitro* when diiodotyrosine is incubated in solution at physiologic pH, and to a greater degree when iodinated casein is incubated at somewhat higher temperatures. It ap-

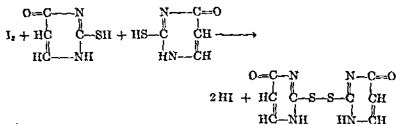


Figure 2

pears that thyrotropin accelerates this reaction *in vivo.* As might have been anticipated, the rate of formation of thyroid hormone under either of the *in vitro* conditions is accelerated by mild oxidizing agents The fact that this final step is an oxidative one naturally raises the possibility that antithyroid compounds might act at this point, but no direct experimental proof of this is available. There is ample proof that the first step, that is, the organic binding of iodine is inhibited by antithyroid compounds, and it is, therefore, unessential to assume that they also act in another place.

Clinical experiments may be performed with radioactive iodine

which illustrate the actions of thiocyanate and antithyroid compounds If a dose of radioactive iodine is given by mouth and serial counts at frequent intervals are made with a Geiger counter placed over the thyroid gland, one can follow the slow and regular accumulation of iodine in the thyroid gland, a process which requires 24 to 48 hours to reach completion. Many weeks are required for this iodine to leave the thyroid gland. It is presumably incorporated into thyroid hormone, and, as the store of hormone within the gland is large, only a very small fraction of the total store is secreted each day. Furthermore, some of the hormone that is secreted is broken down in the tissues and the radioactive iodine contained therein again becomes available to the gland and is re-incorporated in it.

The effect of an antithyroid compound is best shown by first administering the radioactive iodine, and then administering a single dose of the antithyroid agent after the pattern of uptake has been established. When this dose is large enough, the accumulation of radioactive iodine in the thyroid region ceases in about half an hour, and no further accumulation occurs until the effect of the antithyroid compound has worn off. The cessation of uptake caused by the antithyroid compound is a reflection of inhibited organic binding of iodide This phenomenon has provided a useful method for testing the efficacy of antithyroid compounds in man and for determining their duration of action (53).

If, under these circumstances, thiocyanate should be given, instead of the antithyroid compound, very little inhibition of iodine accumulation in the thyroid region is detectible. Without further information, this observation would lead one to believe that thiocyanate ion does not inhibit the accumulation of iodine in the thyroid gland. Actually, however, it shows that the iodide-concentrating mechanism is not the limiting factor in the rate of total iodine accumulation in normal persons Presumably, the iodide-concentrating mechanism is an adaptation which permits the thyroid to function normally even when the iodide concentration of the body fluids is very low. If the blood iodide is not markedly reduced, sufficient iodide can enter the gland by simple diffusion to permit hormone synthesis to proceed at a normal rate This theory would explain why thiocyanate goiter is more prevalent in iodine-deficient regions, and why the goiter due to thiocyanate can be prevented by the administration of iodine

The true action of antithyroid compounds and of thiocyanate ion can be brought out by performing the experiment in a different way. If, in normal individuals, the organic binding of iodine is completely prevented by a large dose of an antithyroid compound given before administration of the radioactive iodine, there will be a prompt but limited accumulation of iodine in the thyroid region This small accumulation of radioactivity in the thyroid region reaches a maximum in about 2 hours and thereafter tends to fall slowly (54) (Fig. 3). If the antithyroid medication is repeated and continued, the radioactivity steadily declines and disappears within a few days. If, after the radioactive iodine has reached a maximum, a large dose of ordinary nonradioactive iodine be given, then there is a rapid loss of the radioactive material from the thyroid region. This strongly suggests that the iodine accumulated in the thyroid gland under the influence of a full dose of antithyroid substance accumulates there and remains in the form of iodide ion. Were it not iodide or a form which is in rapid equilibrium with iodide, the administered nonradioactive iodine would not so quickly displace it This type of evidence lends strong support to the view that antithyroid compounds prevent the organic binding of iodine, which must mean that they prevent the oxidative conversion of iodide to iodine

A very similar result is observed when thiocyanate is given instead of iodide ion. The radioactive iodine which has accumulated under the influence of the antithyroid agent is rapidly and almost completely eliminated from the thyroid region, showing that the action of thiocyanate in man resembles in all respects that in rats, as described by VanderLaan and VanderLaan (58). The fact that large doses of ordinary iodide have an effect similar to thiocyanate in causing a disappearance of radioactive iodide from the thyroid gland does not necessarily mean that these two substances act in the same way. Indeed, there is every reason to believe that their modes of action differ The large doses of ordinary iodide so greatly increase the total iodide-ion content of the body that only a small fraction of it can be contained within the thyroid gland. If the radioactive iodine in the body is also in the form of iodide ion, it will mix with, and label, all of the body's iodide The disappearance of radioactive iodine from the thyroid region following a large dose of potassium iodide merely indicates that prior to the potassium iodide administration a large proportion of the body's total iodide was contained

in the thyroid gland, while afterward the major problem of the iodide was outside the gland. However, it is not yet known whether these phenomena are in any way related to the therapeutic effect of iodine in hyperthyroidism.

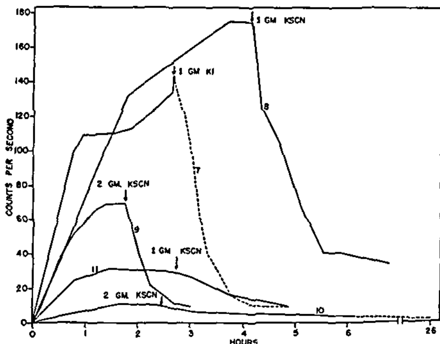


Fig 3 Course of accumulation and discharge of radioactive iodide from the thyroid region in two normal subjects (curves 10 11), and in three patients with hyperthyroidism (curves 7–9), as determined by an externally placed Geiger counter (54) A single dose of 100 mg of mercaptoimidazole was given before the 0 1 millicurie dose of radioactive iodine Note that in hyperthyroidism a much larger amount of iodide is concentrated in the thyroid Both thiocyanate and a large dose of nonradioactive iodide cause a rapid discharge of the radioactive iodide from the gland

The greater capacity of the hyperplastic thyroid gland to concentrate iodide, which has been observed in rats, also holds true for man. If an individual suffering from hyperthyroidism is given a dose of radioactive iodine after administration of an antithyroid compound, the concentration of iodide in his thyroid region is much greater than in that of a normal person (54) (Fig 3). Indeed, this

difference between the hyperplastic and normal gland, as thus revealed, is of greater diagnostic value than the rate of iodine accumulation in the thyroid region of otherwise untreated individuals In hyperthyroidism, a truly remarkable quantity of iodide ion concentrates within the thyroid gland. Often, within $1^1/_2$ hours after oral administration of radioactive iodine the iodide accumulation reaches a maximum, and one can roughly calculate that as much as one-third or one-half of the administered dose of radioactive iodine has entered the thyroid gland in that short interval. This very large quantity of iodide is also readily displaced by a large dose of ordinary iodine, and is easily discharged by thiocyanate.

When used as a diagnostic procedure, the technique of preceding the radioactive iodine by a full dose of an antithyroid agent has the further advantage that repeated tests may be done while the patient receives uninterrupted antithyroid therapy. Nor is there much risk of local radiation effects, since the radioactive material remains in the thyroid for such a brief time.

It is now well established that while antithyroid compounds inhibit the synthesis of thyroid hormone they have no effect upon the activity of the hormone already formed and stored within the thyroid gland. The relatively prompt response of hyperthyroidism to antithyroid medication is due to the fact that in untreated hyperthyroidism there is a rapid formation and secretion of hormone and very little storage. Inhibited synthesis is therefore soon reflected in a decreased rate of hormone secretion. On the other hand, when there is a large nodular goiter, or when iodine has been used for therapy, there is often a very large amount of hormone stored within the gland, consequently, there may be a long delay before antithyroid therapy can influence the metabolic rate. Similarly, the normal thyroid gland contains many months' supply of hormone, and antithyroid medication has no metabolic effect until this store has been used up

Antithyroid Compounds That Have Been Used in Man

RELATIVE ACTIVITIES

Several groups of investigators have tested large series of chemical compounds for their antithyroid activity in rats, and by now assays on well over 600 compounds have been performed Similar

comparisons have also been made in other species, such as the chick These studies have revealed antithyroid activity in more than 200 compounds. By comparison, relatively few substances have thus far been tested for their efficacy in human hyperthyroidism. Those which have received at least a limited trial are listed in Table I.

TABLE I

Relative Effectiveness of Compounds Used in Treatment of Hyperthyroidism*

Compound	Relative effectiveness in *hyperthyroidism*†	Relative effectiveness in normal human subjects—radioactive iodine test *(53)*
6-Methylthiouracil	>1 (51,55), 2-3 (63), 1 (36)	2.
6-Ethylthiouracil	5 (4)	1.
6-Cyclopropylthiouracil	2 (62)	1.
6-*n*-Propylthiouracil	5 (4), 4 (9), 4 (64), 3 (45), <2 5 (25), 2-3 (27,28), 1 (12)	0 75
6-*n*-Butylthiouracil	—	0.75
6-Benzylthiouracil	—	0.75
Thiobarbital	12 (6), 2 (2)	2.
Thiourea	1 (19,14,13)	1.
Diethylthiourea	<1 (60)	—
Tetramethylthiourea	1 (60)	—
Mercaptoimidazole	5-10 (54)	10.
Mercaptobenzimidazole	0 75 (62)	2 5
Aminothiazole	>1 (39), 1 (36), 0 5 (62)	2.5
2-Mercapto-5-amino-1,3,4-thiadiazole	—	2.
Sulfadiazine	—	<0.05
p-Aminobenzoic acid	1[illegible](10), <0 1 (62)	—

[illegible] activity of 1. The ac- [illegible] of various observers [illegible] ne in normal human [illegible]

After several compounds had been used in man, it became apparent that their relative effectiveness did not agree at all well with their potencies, as determined by rat assay Evaluation of the activity of a compound in human hyperthyroidism is a most difficult task, for the rate of response to treatment varies widely in different individuals, and it is almost impossible to say in any given instance whether a slow response is due to inadequate dosage or to some

other factor. Consequently, the rate of response of the hyperthyroid individual to different doses is almost valueless for comparative purposes. The comparison of different doses or of the same dose of different compounds on the basis of effectiveness for maintenance therapy would at first sight seem to be better. Even here, however, there are difficulties. Some individuals, after prolonged therapy, seem to require only minute amounts of an antithyroid compound for complete control, while others need many times as much. Furthermore, as treatment continues, the majority of individuals gradually approach a stage at which no antithyroid therapy is required. Since, short of stopping all therapy, there is no way of telling when this stage has been reached, one might often be misled into thinking that a small dose is exerting an effect when actually the test subject is already well. Probably, the best clinical method of evaluating different compounds is to determine the dose necessary to induce early myxedema This state is readily recognized, and as will be discussed below, some degree of hypothyroidism is probably beneficial to the patient The procedure, however, is time consuming, and even this rigid criterion of effectiveness suffers the disadvantage of a considerable individual variation in the dosage required to induce myxedema

A beginning has been made in the use of radioactive iodine for purposes of evaluating the relative effectiveness of antithyroid compounds in man (53) The method used is that described in a preceding section, and the relative activities of the compounds which have been used for treating hyperthyroidism are listed in Table I. It is evident that the clinical estimates of activity and those arrived at by the use of the radioactive iodine method are not in perfect agreement, but the correlation between these two estimates is much closer than between either of the two human tests and that performed in the rat.

Bearing in mind the difficulties and inaccuracies involved, one may attempt to evaluate the relative antithyroid activity of the several compounds that have been used in therapy. In some instances, observers have specifically defined their impressions of relative activity, while in others these can only be inferred from their published data (Table I).

Thiouracil, which has had the most extensive clinical trial, might usefully be regarded as a standard to which the activities of other

compounds are compared. Unfortunately, there have been very few attempts to define the minimal effective dose either for initial treatment, for maintenance, or for the induction of early myxedema. The dosage most commonly used for the initial period of treatment has been 600 mg daily. Many observers used larger doses during their earlier experience with this form of treatment, and later reduced the dose to 600 mg. a day Few observers have used smaller dosages, but some have found that 200 to 400 mg. daily, in divided doses, is adequate in many, if not all, instances It would appear that 600 mg. a day is really an excessive dose; there are rare instances, however, where smaller amounts have not been effective. For purposes of comparison with other compounds, one might arbitrarily settle on 400 mg. as the maximal dose of thiouracil needed for initial therapy. The maintenance dose has usually been stated to fall between 50 and 200 mg a day. Certainly very few cases require more; and when less than 50 mg daily is given one is frequently uncertain whether any at all is required From the limited data available as to the dose required to induce myxedema, it would seem that 200 or 300 mg. a day is the least that will achieve this result with any degree of regularity. Many observers have found that if the initial dose of 600 mg. daily, or more, is continued after metabolic equilibrium has been achieved, myxedema will result in almost every instance. With these figures in mind, one might then assign thiouracil an activity of 1 and attempt to arrive at a figure for activity of the other compounds that have been used

Methylthiouracil, which has been extensively used in Europe and especially in Scandinavia, appears to be more active than thiouracil Most workers who have compared the two have concluded that it is distinctly more active (29,40,55,63); perhaps one might estimate that it has an activity approximately twice that of thiouracil Despite its apparently greater activity, the majority of reports to date cite the use of about 600 mg. a day, and many workers have used larger amounts. It would appear, however, that myxedema has been much more common when this is done; for example, Frisk (17), using doses of 500 mg daily, noted an elevation of the serum cholesterol to values above normal in almost every instance, and to very high levels in a considerable number of his cases. Similarly, thyroid enlargement was seen in half of the patients. Most observers have found that the maintenance dose is distinctly smaller with

methylthiouracil, and seldom is more than 100 mg. daily required. In several clinics, as little as 25 mg. a day has seemed to be effective. The clinical impression that methylthiouracil is about twice as active as thiouracil is supported by the radioactive iodine assay in human beings, where a similar ratio was observed. Five other 6-substituted thiouracils have been tested in human hyperthyroidism, but none except the *n*-propyl derivative has been extensively investigated.

6-n-Propylthiouracil has now been used as widely as the methyl derivative. Earlier tests with very small dosages showed that some cases responded well to as little as 50 mg. daily in divided doses. Many cases required larger doses, but in very few instances was more than 150 mg daily required (4) As experience with this substance accumulated, it became apparent that certain patients failed to respond to these small doses and that 300 mg. daily was needed in some cases for complete control of the hyperthyroidism It has recently been observed that an occasional patient fails to respond completely even to this larger dose (12). This wide range in dosage has sometimes given rise to the impression that there is a greater individual variation in the response to this substance than to thiouracil. This may not be true, however—no comparable studies have been published on the effectiveness of very small doses of thiouracil or methylthiouracil This lack of comparative data makes it difficult to assign an activity value for propylthiouracil If one were to accept the evidence of the first publications dealing with this matter, the activity might be estimated at 2 to 5 times that of thiouracil (4,9,25,27,28,45,62,64) However, when the evaluation is based on later studies and the occasional case that does not respond fully to 300 mg is taken into consideration, the radio activity appears to be considerably less It would also appear from the limited data published that the maintenance dose and the dose required to produce myxedema is no smaller with propylthiouracil than with thiouracil. From this, one could assume that the activity of propylthiouracil and of thiouracil are about equal, a conclusion which receives support from the radioactive iodine measurements. These measurements show propylthiouracil to be slightly less active than the parent substance

6-Ethylthiouracil has not been extensively investigated, and its activity seems not to differ from propylthiouracil Williams (62)

compared a series of thiouracil derivatives on the same patients, and concluded that *6-n-propylthiouracil*, *6-cyclopropylthiouracil*, and *6-n-butylthiouracil* are about equally effective and approximately twice as active as thiouracil. Isopropylthiouracil, tertiary butylthiouracil, and benzylthiouracil have been administered to man, but their relative activities have not yet been determined. A few investigators have employed *thiobarbital*, and there is general agreement that it is more active than thiouracil (2,6). The estimates of activity, however, vary, ranging from 2 to 12 times that of thiouracil. The smaller figure would agree with the data obtained with radioactive iodine.

Thiourea, though it has been used for several years, has not received the same extensive clinical trial as thiouracil and its derivatives. It has usually been given in large doses—1 to 3 Gm. a day; in a few instances, as much as 9 Gm (41) have been given. Hercus and Purves (20) found that 0.4 to 0 6 Gm. daily is sufficient, and more recently a preliminary trial has been made with even smaller doses (14,66); this study indicates that definite effects can be observed from as little as 210 mg. daily.

Williams (60) has tested the *diethyl* and *tetramethyl* derivatives of thiourea. The former compound he abandoned because of toxic effects and low activity; the latter he found to be as active as thiouracil. *Allylthiourea (thiosinamine) is apparently active, but like* the diethyl derivatives, too toxic for human use (20) Two imadazole derivatives have received limited attention. *2-Mercaptobenzimidazole* was found to be somewhat less active than thiouracil (62), while *2-mercaptoimidazole* appears to be highly active in man (53,54) These two compounds gave values of 2 5 and 10, respectively, in the radioactive iodine test. *2-Aminothiazole* was introduced by the French during World War II (39), and it was found to be distinctly more active than thiouracil. Williams (60) failed to confirm this high activity, but the radioactive iodine test showed an activity of 2.5. *2-Mercapto-5-amino-1,3,4-thiadiazole* has been tried on a few patients, without conclusive results. It appeared to be twice as active as thiouracil when tested in normal human subjects. *Sulfadiazine* and *potassium thiocyanate* have been extensively employed in man *for other purposes, but there are no reports* indicating that they are effective in hyperthyroidism. Sulfadiazine has been given for prolonged periods to a great number of patients,

were the compound active in this regard in man, one might expect that a thyroid effect would have been detected. To date, however, neither clinical hypothyroidism nor goiter formation has been attributed to this compound. The radioactive iodine test has demonstrated an absence of antithyroid activity in the largest dose used. Potassium thiocyanate is not an antithyroid agent in the strict sense, but it has a marked effect on the iodide-concentrating mechanism. Apparently, inhibition of this mechanism is not an effective treatment for hyperthyroidism, though goiter and early myxedema have frequently been observed in patients being treated for hypertension. Berman (10) has claimed that *p-aminobenzoic acid*, which like sulfadiazine is slightly active in rats, is effective in hyperthyroidism when given daily by intravenous injection. Williams was unable to find any activity when this substance was given in large doses by mouth.

RELATIVE INCIDENCE OF SIDE EFFECTS

The most important single factor which determines the superiority of one compound over another is the incidence of untoward reactions. A great deal is known about thiouracil in this regard, and it is therefore convenient to compare the side reactions of other compounds to those of thiouracil.

The extensive study by Van Winkle *et al.* (59) analyzed the incidence of side effects from thiouracil in 5,745 cases. In this series, febrile reactions occurred in 2.7 per cent and agranulocytosis was diagnosed in 2 5 per cent of cases. The over-all mortality due entirely to agranulocytosis was 0 4 per cent Moore (35), in an analysis of 1,091 cases, recorded fever in 4 9 per cent, agranulocytosis in 1.74 per cent, and a death rate of 0 46 per cent A review of 2,049 cases which had appeared in the literature up to that time showed that fever occurred in 2 7 per cent of cases, agranulocytosis in 1 5 per cent, and death in 0 3 per cent Numerous other, less common reactions, have been described. Many of them may be regarded as variants of the drug fever syndrome, they include urticaria, various skin rashes, arthralgia, gastrointestinal symptoms, swellings of the face or feet, lymphadenopathy, and Mikulicz's syndrome These may occur with or without fever. Occasional reports have cited headache, diarrhea, jaundice, and other bizarre symptoms, but it has

never been established whether these can be due to thiouracil. The most important reaction, of course, is agranulocytosis, and it is a serious drawback to this particular compound even though the condition can now be treated with antibiotics. Almost as important as agranulocytosis is the tendency to sensitivity reactions of such severity as to make it impossible to continue the thiouracil treatment.

It would appear from the literature that about 5 per cent of patients react either by disconcerting changes in the leukocyte count or by some variants of the drug fever syndrome, making further treatment with thiouracil impossible The other antithyroid agents which have given rise to troublesome side effects have in general followed the pattern set by thiouracil Apparently, however, there are some differences, worthy of mention. Thiourea has caused agranulocytosis and drug fever, but it seems more prone to give rise to malaise, skin eruptions, and gastrointestinal upset than is thiouracil It also tends to cause anosmia, and it imparts an unpleasant odor to the breath Thiourea is eliminated slowly from the body; the high incidence of toxicity when large doses are employed may be due to cumulative effects. It is certainly true that there is a relation in this instance between dosage and the incidence of side effects In a series of 54 cases, Danowski, Man, and Winkler (14) noted only two significant toxic reactions when doses of 0 28 mg. or less were given. When doses in excess of 1 Gm daily, were employed, some form of reaction occurred almost invariably This relationship between dose and toxicity is important because some authors have expressed the view that the dose of thiouracil bears no relationship to the probability of side effects Such an idea is, of course, unreasonable, and there are numerous instances of a greater tendency for sensitivity reactions to occur when a drug is given in a large dose It is clear that thiobarbital is more prone to cause side effects than is thiouracil, and in this instance, too, excessive doses caused unpleasant symptoms in almost every instance (2) Agranulocytosis was observed, however, even when the dosage was small (6)

Most observers who have compared thiouracil and methylthiouracil in equal dosage have found that the methyl derivative is less prone to give rise to sensitivity reactions than is thiouracil (12,26,-55,63). However, several cases of agranulocytosis have been reported, some of them fatal (23). Propylthiouracil seems to be unusually well tolerated. To date, the literature contains two or three

references to drug fever, and 2 cases of agranulocytosis, but no fatalities. In our own series of 250 cases, there have been several instances of rash, some of them probably due to propylthiouracil. But, with one possible exception, the drug has not had to be withdrawn because of toxicity. The possible exception was a patient in whom a rash occurred; treatment was changed to another compound without determining whether propylthiouracil was responsible for it. Williams (62) treated 39 patients with propylthiouracil without any significant reaction, and 58 patients with cyclopropylthiouracil, also without mishap. He also found 6-isobutylthiouracil to be well tolerated in 43 cases, with itching of the skin as the only side effect. Most observers are now agreed that 2-aminothiazole is more toxic than thiouracil (36,62). In addition to causing agranulocytosis and drug fever, it has seemed to be associated with liver disease and other severe systemic reactions The two imidazole derivatives which have been tested appear to differ widely in their tendency to cause sensitivity reactions Williams, in a series of 17 trials, found that 2-mercaptobenzimidazole gave rise to 5 instances of fever, 2 of them associated with leukopenia Preliminary trials with mercaptoimidazole have indicated that it is well tolerated by man (52).

Diagnosis of Hyperthyroidism

Since the introduction of newer methods of treatment for hyperthyroidism, the matter of diagnosis has become considerably more important than heretofore Now that treatment can so readily be carried out, the recognition of hyperthyroidism in its mildest forms has become important But even in clinics devoted especially to the study of thyroid disease, it is frequently difficult to arrive at a diagnosis, for hyperthyroidism may manifest itself in hardly recognizable forms

Symptoms. The typical symptoms of hyperthyroidism require no special comment, but some of the variations which are commonly encountered are of interest Weight loss, which is such a common accompaniment of this disorder, sometimes may not occur even in the face of severe hyperthyroidism Very marked weight loss sometimes indicates that some complicating disorder is present; for example, in some instances loss of weight is first noticed at the onset of diarrhea or loss of appetite It is sometimes difficult to obtain a proper dietary history, but when this can be done the increased

food intake becomes apparent. Persons with hyperthyroidism occasionally complain of poor appetite, but at the same time they may eat twice as much as a normal person. Heat intolerance is a symptom seldom complained of, but it is important to evaluate heat intolerance in those individuals who complain of sweating. The most difficult symptoms to evaluate are those related to nervousness, and the differential diagnosis of the cause of nervousness on the basis of symptoms alone is often not possible Typically, of course, the nervousness of an anxiety neurosis tends to occur in spells, and the attacks come on without adequate precipitating cause. The nervousness of hyperthyroidism tends to be constant, though it may become worse under conditions of stress or excitement. Palpitation, provided it does not occur only in isolated spells, is a suggestive sign of hyperthyroidism. Ready fatigue and shortness of breath on exertion are common symptoms, and it is usual for the fatigue to become worse as the day wears on. Many people with hyperthyroidism sleep poorly, some complain of headache, others of eye trouble.

Eye Signs The diagnosis is usually very easy when the typical eye signs of Graves' disease are present, for no other disease gives rise to these typical phenomena In examining the eyes one should watch for evidence of lid retraction, evaluate the appearance of stare, and ascertain whether there is demonstrable weakness of the external ocular muscles One must not be led astray, however, by the condition of familial or racial largeness of the eyes With advanced age, there may be difficulty in looking upward, so that this sign alone is not conclusive The appearance of the eyes in severe hypertension may also suggest hyperthyroidism. A previous attack of thyrotoxicosis, which may have passed off spontaneously or have been treated, might leave an individual with fairly typical eye signs of Graves' disease

Means (31) has drawn our attention to cases of Graves' disease in which the eye signs are so out of proportion to the mild hyperthyroidism that the latter component may not be recognizable. However, it would seem appropriate under these circumstances to make the diagnosis of Graves' disease and treat the condition accordingly

Goiter The presence of a goiter is often cited as one of the signs of Graves' disease Actually, it is probably better to place little reliance on the size of the thyroid gland when attempting to arrive at a diagnosis of hyperthyroidism. Simple goiter is far more common

than hyperthyroidism, and in many cases of hyperthyroidism it is difficult to detect thyroid enlargement. This is especially true in men, in obese women, and in individuals with short necks The presence of a bruit in an enlarged thyroid is probably more significant We have come to regard a bruit as a sign of thyroid hyperplasia; while hyperplasia is present in a newly developing goiter from whatever cause, one seldom finds a bruit in the simple goiters encountered in this country, and a bruit in the thyroid is strongly suggestive of hyperthyroidism. To be helpful in this regard, the sound must be loud and should be audible throughout most of the cardiac cycle Faint or transient sounds over the thyroid region are of no significance.

Hypermetabolism. Other helpful signs of hyperthyroidism are a generalized motor overactivity, a rapidity of movement and speech, and tremor. The tremor is not limited to the outstretched fingers, and other muscle groups are often tremulous, especially when the individual is under tension or upset Probably the most useful signs of hyperthyroidism are those related to excessive heat production Typically, the skin is flushed, warm, and moist. The hands are almost invariably warm and moist The increased circulation through the skin and extremities gives rise to a bounding pulse, often to capillary pulsation, and a widening of the pulse pressure

Evaluation of the individual's heat intolerance is most helpful. By taking into account the temperature of the room and the amount of clothing worn, the examiner can often judge whether the patient is uncomfortably warm, as compared to himself Useful information in this regard can be obtained by inquiring about conditions at home, such as the thermostat setting or the quantity of bed clothes In keeping with the increased peripheral circulation, the heart action is more vigorous in hyperthyroidism. Often, a large area of the chest and upper abdomen can be seen to pulsate with each heart beat. The forcefulness of the cardiac impulse transmitted to the hand when pressed firmly over the precordium is one of the most reliable indices of the presence or absence of hyperthyroidism.

Laboratory Tests. The special tests for hyperthyroidism are a help in arriving at a diagnosis in doubtful cases. They are not as important, however, as the clinical picture. Determinations of the basal metabolic rate are very widely done, but it is probably true that in the majority of instances the tests are of little value. Cer-

tainly, a single reading taken in a doctor's office is meaningless. Those who have used this test extensively agree that reliable values can only be obtained if the test is repeated at frequent intervals, and one may have to do three or four tests on consecutive days in order to achieve a proper evaluation of the metabolic rate. It is most useful to examine the respiratory tracings, for the presence of the well-recognized types of irregularity might show that the test was not reliable. If the determination of the metabolic rate is done under careful basal conditions, normal individuals will show rates which vary from zero to —25 per cent. In view of this wide range, it is quite possible for the metabolic rate to be zero in clinically obvious hyperthyroidism. Markedly elevated rates, of course, can be due to other things, such as severe hypertension, leukemia, infections (even though the temperature may not be elevated), and in various forms of malignancy, particularly lymphoma and carcinoma of the lung. Certain very obese people have high metabolic rates without other evidence of thyroid disease, and the commonest cause of an elevated rate is, of course, a poorly performed test in an agitated person.

The level of serum cholesterol is of little value in the diagnosis of hyperthyroidism, unless it be very low. The cholesterol level might also be markedly reduced by chronic wasting disease and by severe anemia This test is of value, however, in following the course of therapy, since in any given individual the level remains quite constant under normal circumstances

Chemical determination of the concentration of protein-bound iodine in the serum or plasma has been proposed as a useful method in the diagnosis of thyroid disease The range of normal values apparently varies according to the technic used, but seems to be quite wide The concentration may be greatly increased in hyperthyroidism, however, hyperthyroidism may also exist without a concomitant elevation of the protein-bound iodine beyond the normal range

The use of radioactive iodine in the diagnosis of hyperthyroidism is now coming into wider use Various technics are currently being employed to test the activity of the thyroid gland Most workers measure the urinary excretions of a dose of radioactive iodine In the presence of a highly active thyroid gland, much of the iodine is accumulated in the thyroid and fails to appear in the urine, so that urinary excretion bears an inverse relation to the intensity of thyroid function.

A method which is simpler and which may prove to have wider application is the direct measurement of radioactivity over the thyroid region. Here the rate of iodine accumulation can be measured in the course of a few hours. Two variants of this technic appear to have merit. In the first, radioactive iodine may be given alone and measurements made over the thyroid region for 2 or 3 hours. From these values one may predict fairly accurately the entire course of the iodine uptake. The rate of accumulation under these conditions is greater in hyperthyroidism than in normal individuals, though some overlap is occasionally observed (3)

A second, and perhaps better, method is to measure the iodide accumulation In this test a full dose of an antithyroid compound is first administered and 30 to 60 minutes later a dose of radioactive iodine. Within 2 hours, the accumulation of iodide in the thyroid region reaches a maximum, and the height of this maximum seems to correlate well with the intensity of thyroid hyperplasia It seems likely that within the next few years a sufficient quantity of data will be accumulated to permit an evaluation of the usefulness of radioactive iodine in the diagnosis of thyroid disease

The use of the protein-bound iodine method and of procedures employing radioactive iodine are limited to special clinics with the proper facilities. They will probably never supplant the more useful clinical criteria for diagnostic purposes

Plan of Treatment

If hyperthyroidism is to be treated with an antithyroid compound, there are two immediate aims in view The first is to restore metabolic equilibrium as quickly as possible, the second is to conduct the future course of therapy in such a way as to achieve the longest possible remission

The initial phase of treatment has received considerable attention The proper dose, frequency of administration and factors which modify the rate of improvement are now fairly well understood But the best course to follow after the hyperthyroidism has been controlled has not yet been determined Before starting treatment, one should have an accurate record of all the manifestations of the illness, together with the results of as many special tests as are practicable This record can then serve as a point of reference in

evaluating the response to treatment and the significance of any symptoms or signs which are not completely eliminated by the therapy. The decision as to whether or not to restrict the patient's activities rests largely upon the severity of the illness and the nature of

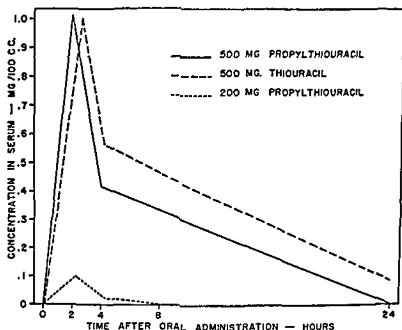


Fig. 4 Thiouracil and propylthiouracil concentrations in the plasma after oral administration of single doses (10a) A detectable quantity was present in the serum for only 4 hours following administration of 200 mg. of propylthiouracil With a 500 mg dose, thiouracil could be detected at the end of 24 hours, but propylthiouracil could not

the complications It certainly seems best not to restrict activities unless it is absolutely necessary, and it is usually good practice to permit the patient to do as much as his well-being dictates. Persons with hyperthyroidism often find it difficult to rest, so that unnecessarily enforced inactivity may be quite uncomfortable. Only the severest cases, and those with cardiac complications or extensive muscular wasting, need discontinue their usual occupation. Even when physical activity must be restricted, the patient can usually

be handled at home, and it is very seldom that bed rest need be enforced.

The initial dose to be employed depends, of course, upon the compound used. In the case of propylthiouracil, 300 mg. a day appears to be optimal. When this compound is used, it should be given in divided doses because of its rapid elimination from the body (53) (Fig. 4). This may be most easily accomplished by having the patient take 100 mg every eight hours, for example the first dose on arising in the morning, the second in the midafternoon, and the third at bedtime. It would be of advantage to have available a compound with a more prolonged action, so that a single daily dose would suffice. This appeared to be practical with thiobarbital, and it seems that mercaptoimidazole might be used in this way. The initial dosage should be continued until all signs and symptoms of hyperthyroidism have completely disappeared. To make sure that this aim is achieved, one should attempt to continue the initial dose for as long as possible, reducing it only if clear evidence of hypothyroidism appears, or if marked thyroid enlargement is observed. When this stage has been reached, the dose might be cut in half, so that 50 mg. are given at 8 hour intervals. Subsequent reductions in dosage should be made only if hypothyroidism or thyroid enlargement demand it

Rate of Response. The initial observation (42) that previously untreated cases of typical Graves' disease respond at a rate similar to that observed when iodine was given has been confirmed by several observers. In some series, the average rate of response has been somewhat slower than that following iodine. It is clear that the severity of the disease bears no relation to the rate at which the basal metabolic rate falls, but, if the initial level is high, a longer time is, of course, required before the rate becomes normal. The two factors which significantly delay the rate of response, although not invariably, are previous iodine medication and the presence of a large nodular goiter. If iodine has been given for a short time only, and partial improvement has occurred, a further and immediate response is often seen when an antithyroid compound is substituted for the iodine In other cases, especially when iodine has been taken for long periods, several months of constant antithyroid treatment may be required before a metabolic response is observed In certain patients who have had iodine, a change in therapy from iodine to an

antithyroid compound is followed by a transitory exacerbation of the disease. This would imply that the hormone stored under the influence of iodine treatment is released at an increased rate when the iodine is stopped, and control is only achieved when this store has been largely used up. The very long delay which may occur in iodine-treated cases is a serious matter in some instances. Any physician who prescribes iodine for the treatment of hyperthyroidism should do so in full realization that should it fail, a difficult therapeutic problem will present itself.

When the hyperthyroidism is severe, it is probably best to withdraw the iodine slowly over several weeks after antithyroid therapy is begun. In mild cases, the iodine can be withdrawn abruptly and the antithyroid compound either started at once or withheld until the symptoms return

Initial Dose What the initial dose of an antithyroid compound should be is a difficult question to answer. Ideally, it should be an amount which will bring about a complete remission in all patients in a minimal period of time. It is clear that there is nothing to be gained by giving doses in excess of this, but some have felt that it is better to start with a small dose and increase it gradually if need be. One important fact has emerged during the past few years relating to the size of the initial dose, namely, that a sufficient amount of medication should be given to control completely all manifestations of the disease If this is not done, the chances of a lasting remission appear to be significantly reduced It is therefore probably wise to continue the initial dose until the signs of early myxedema appear, and then reduce dosage to the maintenance level so that the patient will remain in a state of normal or slightly subnormal thyroid function for several months

Length of Maintenance Therapy. The proper duration of the maintenance period has not yet been defined So far, we cannot tell when the treatment can safely be withdrawn, short of stopping it altogether to see whether symptoms return. As it is the aim of treatment to achieve a state of complete remission of the disease, it might be instructive to analyze the features that may contribute to the incidence of lasting remission (Table II) Most workers who have treated large series of cases have concluded that the duration of treatment is a factor of considerable importance When treatment was continued for less than 6 months, the incidence of remission

TABLE II

Varying Incidence of Remission of Thyrotoxicosis after Antithyroid Therapy, as Reported from Sixteen Clinics*

Authors	Treatment	No of cases completing therapy	No of lasting remissions	Remission rate, per cent
Palmer (38)	Thiouracil	9	4	44
Barr and Shorr (5)	Thiouracil, usually for more than 6 months	48	37	77
Williams (61)	Thiouracil, prolonged treatment	100	49	49
Hadorn and Beer (18)	Methylthiouracil	10	6	60
Frisk (17)	Methylthiouracil, 1-24 mos	29	24	83
Beierwaltes and Sturgis (8)	Thiouracil, 3-16 mos	13	8	62
Wilson (63)	*Thiouracil and methylthiouracil*, 1-14 mos	18	3	17
Meulengracht *et al* (32)	Methylthiouracil, 8-9 mos	60	55	93
Hercus (19)	Thiourea and thiouracil	23	20	87
Reveno (44)	Thiouracil, average of 13 mos	7	16	—
Aranow *et al* (1)	Thiouracil, 6 mos or more			
	Primary toxic goiter	14	8	57
	Recurrent hyperthyroidism, postoperatively	19	4	21
Poate (40)	Thiourea, thiouracil, methylthiouracil 3-4 months of "minus metabolism"	183	159	87
Himsworth *et al.* (21)	Treated for 6-24 mos	25	18	72
Rose and McConnell (48)	Thiouracil, prolonged treatment	29	16	55
Bartels (7)	Thiouracil, 1-21 mos			
	Primary hyperthyroidism	9	5	56
	Recurrent hyperthyroidism, postoperatively	12	3	25
Winkler, Man, and Danowski (66)	Iodine, small doses of thiourea	10	2	20

* In most instances the figures represent the earlier experience with this form of therapy and are now subject to revision

was almost always distinctly less than when prolonged therapy was given. Probably a factor of greater importance than the duration of treatment is the degree to which hyperthyroidism is controlled One gains the impression from some publications that the investigators were content if marked improvement was induced There are relatively few reports which define clearly the state of thyroid function which was maintained during the latter months of therapy. In some

published series the incidence of myxedema, of thyroid enlargement, or of markedly elevated serum cholesterol values, has been high; these groups apparently attained a higher remission rate than did some others. Thus, Barr and Shorr (5) noted 5 instances of myxedema, and 78 per cent of their series of 48 patients in whom therapy was discontinued experienced lasting remissions This might imply that, on the whole, their cases were thoroughly controlled and that this factor may explain the satisfactory end results.

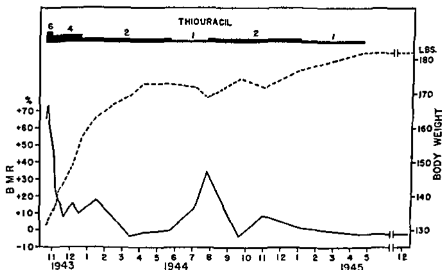


Fig 5 Course of treatment with thiouracil of a patient with severe hyperthyroidism of 6 months' duration There was marked exophthalmos, auricular fibrillation, a small goiter, and a weight loss of 33 lbs When, at the end of 6 months, the dose was reduced to 0 1 Gm once daily, signs of hyperthyroidism returned. When treatment was discontinued after 18 months, there was no recurrence, and no signs or symptoms developed during the next 3 years

Poate (40) has called attention to the importance of thoroughly controlling the hyperthyroidism He has found that if the metabolism is kept slightly on the negative side for 3 to 4 months, the remission rate approaches 100 per cent. He suggests giving a small dose of thyroid to prevent full myxedema and continuing a relatively large dose of antithyroid compound during the maintenance period. Table II lists some of the published data on the incidence of lasting remission. In some instances, it is difficult to tell what determines the degree of success achieved. One is led to conclude that the duration of treatment and the completeness of control are the two

most important factors thus far demonstrated. Some workers claim that severe cases are not likely to undergo a remission, but such has not been the experience of the majority of investigators. It has also been claimed that long-standing hyperthyroidism militates against a remission and that men are less likely to recover than women. Patients with large goiters also supposedly show poor end results.

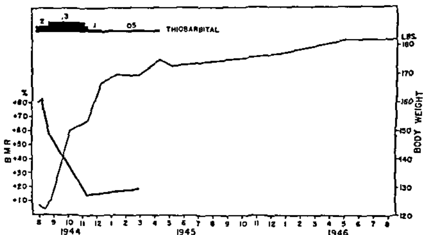


Fig 6 Treatment with thiobarbital of a patient with severe hyperthyroidism of 1 year's duration There was a large goiter, and a weight loss of 40 lbs Iodine had been given for 2 months Though the basal metabolic rate was not once found to be subnormal, there was evidence of early myxedema and a further enlargement of the thyroid during the latter part of the treatment Complete recovery was maintained for 3 years after treatment was stopped; the goiter regressed only partially during that time.

Most of these conclusions have been based on small series of cases, and are not supported by wider experience. The treatments and subsequent courses of two patients are shown in Figures 5 and 6 These are instances of severe hyperthyroidism in men, in one associated with severe exophthalmos, in the other with a large goiter. However, both have remained well for 3 years It is certainly true that some persons with hyperthyroidism suffer repeated relapses, no matter how they are treated Even multiple subtotal thyroidectomies do not control this type of case. If cases are selected on the basis of their resistance to cure by subtotal thyroidectomy, one might expect a high incidence of relapse after antithyroid therapy.

Iodine Medication The question whether iodine medication,

given during the course of antithyroid therapy, influences the rate of cure is one of considerable importance. There is some indication that the concurrent administration of iodine may lower the incidence of remission when antithyroid therapy is discontinued. When iodine alone was used for the treatment of hyperthyroidism, very few cases reached the stage of remaining well when the iodine was stopped. In recent years, only the mildest cases, as a rule have been treated with iodine alone, but even in these it would appear that lasting remissions without iodine have seldom been achieved. This might suggest that iodine therapy is not conducive to remissions. However, the poor results may be due to the fact that iodine seldom induces hypothyroidism and only occasionally controls the disease completely. It is noteworthy that in the series of cases treated with iodine and small doses of thiourea, only two out of ten remained well when the thiourea was stopped (66). In a limited number of observations of our own, patients who received iodine either during or after complete control with antithyroid compounds have tended to relapse more frequently than those who did not. This is an important question which needs to be answered by further investigation. At present, however, it would seem best to avoid iodine during and after treatment with an antithyroid drug.

Criteria for Discontinuing Antithyroid Therapy It would be most important if one could judge wisely when to discontinue antithyroid therapy Stopping medication too soon may possibly lengthen the necessary period of treatment, yet one hesitates to continue treatment for an unnecessarily long interval Some features which seem to be helpful in deciding when treatment can be stopped have been suggested by several workers. A decrease in the size of the thyroid gland is suggestive evidence that the fundamental disorder leading to hyperthyroidism has improved or disappeared Sometimes, however, a decrease in thyroid size occurs early in the course of treatment, and in such cases it does not seem to signify a disappearance of the fundamental disorder Some have suggested that when there is no return of symptoms with a very small maintenance dose, treatment may be safely discontinued This, unfortunately, is not invariably true Probably the best sign that the patient is going to remain well after treatment is stopped is the complete freedom from signs or symptoms for several months. Barr and Shorr (5) have expressed the view that mental or physical trauma or an inter-

current disease may be factors in inducing a relapse after cessation of treatment If this is so, it would suggest that the individual's personal problems and conflicts should receive attention and that he should become suitably adjusted to his environment before treatment is discontinued.

Signs and Symptoms of Recurrence. When treatment is finally withdrawn, it is usually an easy matter to detect a recurrence in its earliest stages The intelligent patient can often tell that symptoms are returning before there are any detectible obvious signs. If the individual has been maintained in a state of slight hypothyroidism, cessation of treatment may be followed by a change in physical signs leading the physician to suspect that hyperthyroidism is returning. But loss in weight, acceleration in pulse rate, or a change in the basal metabolic rate or serum cholesterol may not under the circumstances, mean that the patient has started to relapse. Observation should be continued long enough to make certain that the disorder is actually returning before treatment is resumed; otherwise, therapy may be continued unnecessarily for many months longer. When treatment is resumed, clinical improvement is usually more rapid than during the initial period. Some patients who have experienced repeated relapses after therapy is discontinued learn to resume medication before any detectible signs of hyperthyroidism appear. In such cases of relapsing hyperthyroidism, this plan of treatment would appear to be entirely justified

General Considerations. A few practical considerations about the treatment seem worth being pointed out. The physician and the patient should recognize that each dose of medicine has no immediate effect, and that it is the steady, prolonged action which is important Some physicians have given their patients the impression that they might take the medication whenever they feel nervous or upset, and omit it when they feel well This sporadic therapy may have some psychotherapeutic effect, but certainly the rate of metabolism will not be altered At the beginning of treatment, the patient might well be informed that after a variable period of time he will feel well again, but that he will need to take a smaller dose of medicine for many months in order to remain well He should be made aware of the fact that intermittent therapy is ineffective.

A part of the treatment of hyperthyroidism is doubtless psychotherapy. Improvement is certainly aided by reassurance and im-

peded by anxiety or fright. With thiouracil, the patients had to be told to report without delay any untoward events or symptoms, for prompt detection of agranulocytosis or drug fever, but there was no need to inform them that they were being treated with a medicine which might possibly kill them. If, with each dose that he took the patient thought that he might die, he would not be apt to achieve a state of mental repose consistent with improvement. It almost seems as though some physicians place upon the patient the responsibility of any untoward reactions to the medication. If hyperthyroidism is to be properly treated, the physician must control his own nervousness and apprehension before starting the patient's therapy. The whole attitude of the physician toward hyperthyroidism should change if this therapy is to be used. Hyperthyroidism should be regarded, not as a medical emergency, but as a chronic illness which can only be effectively controlled by prolonged treatment. In view of this, the patient should be assured at the beginning that he will soon be better and that he can look forward to being well eventually. The patient should have the feeling that he suffers from an easily remediable disorder, and that his health is not in grave danger. It is helpful to make the patient aware that the size and shape of the thyroid gland is a minor consideration. He can well be told that when the treatment is completed the goiter may be smaller than it was and may even disappear, but that it is not of any grave consequence if it grows somewhat larger or remains the same size. He might be assured that when his health is regained, the goiter can be removed by surgery, should the appearance of the neck be of sufficient concern to him.

Changes in Size of Goiter

On the basis of animal experiments, one could expect a steadily progressive enlargement of the thyroid gland during therapy, as the metabolism is brought to normal and maintained there The fact that this is not usually observed suggests that there is a striking difference between the action of an antithyroid compound in a patient with hyperthyroidism and the effect of such a substance in a normal individual.

Antithyroid therapy in hyperthyroidism usually results in a gradual *decrease* in the size of the goiter In some instances, there is a slight and transient enlargement during the first few weeks of ther-

apy. In a small proportion of the cases (1 or 2 out of 100) the expected striking enlargement is observed.

It is now well established that if the antithyroid dosage is maintained at a level high enough to induce myxedema, a rapid enlargement of the thyroid gland follows almost invariably, often associated with signs of increased vascularity This same result has been observed after prolonged administration of antithyroid therapy to individuals with initially normal thyroid function. In these, no detectible changes were noted until after many months of treatment with large doses. Then, within a few weeks, signs and symptoms of hypothyroidism, a striking increase in the serum cholesterol and a rapid drop in the basal metabolic rate occur concurrently with the sudden development of a vascular goiter. This observation suggests that the normal human being is no different in this respect from a normal animal in the response to full dosage of an antithyroid compound. It suggests also that after hyperthyroidism has been completely controlled, the response becomes like that of a normal individual, except that myxedema ensues much more quickly. This latter fact is understandable because the thyroid gland in Graves' disease has, initially, a very small store of thyroid hormone which becomes still further depleted after the thyroid has been under the influence of thiouracil for several weeks. Consequently, there is a much shorter latent interval in the induction of myxedema.

Most experimental observations are in keeping with the theory that the formation of a goiter as a result of antithyroid therapy is a compensatory phenomenon The induced deficiency in thyroid hormone is a stimulus to the release of thyrotropic hormone from the hypophysis, which acts upon the thyroid gland and induces hyperplasia and enlargement If this is the mechanism in the normal animal and in the human being with normal thyroid function, one must assume that a different state of affairs obtains in Graves' disease

The fact that thyroid enlargement is not the usual sequel to a marked reduction in the rate of metabolism suggests that the initially elevated metabolism is not brought about by an increased secretion of thyrotropic hormone from the hypophysis. It suggests that the normal pituitary–thyroid interactions are deranged or are not operative in this disorder, and that pituitary stimulation of the thyroid only comes into play when thyroid function is normal or

depressed. A consideration of the 1 or 2 per cent of cases which exhibit a striking enlargement of the thyroid during therapy, even when the hyperthyroidism has not yet been controlled, suggests that an occasional case of hyperthyroidism may indeed be due to excessive production of thyrotropic hormone. It is, of course, usually assumed that the hyperthyroidism of acromegaly is due to a primary pituitary overactivity, and it is not inconceivable that there might be an overactivity of one or another tropic factor in other conditions as well However, even the unusual cases which exhibit a marked thyroid enlargement during therapy show a decrease in the size of the goiter if proper treatment is continued long enough. It seems possible that these cases do not really represent a different type of disorder, but merely exhibit to a more marked degree the transitory thyroid enlargement often seen in the usual run of cases. It is as difficult to evaluate the nature of this temporary slight enlargement as of the later decrease in thyroid size In any consideration of changes in thyroid size, one must bear in mind that there are no available means for accurately recording the size of the thyroid gland. Estimates of changes in thyroid size are made difficult by variations in consistency and in the amount of subcutaneous fat. Similarly, variations in the signs of hyperemia—bruit and thrill, may be brought about by changes in the general systemic circulation rather than by any alteration in the thyroid gland itself.

Complicating Conditions

There are relatively few disorders which may not occasionally coexist with hyperthyroidism, and few of them require special consideration because they do not modify the course of therapy. Special interest, however, attaches to such conditions as pregnancy, the menopausal state, and diabetes

Pregnancy. Most observers have found that pregnancy does not constitute a contraindication to the use of antithyroid drugs Indeed, it would appear that this is the most appropriate form of treatment for pregnant women with Graves' disease It has been frequently observed that simple goiter or hyperthyroidism starts for the first time during or soon after pregnancy It is interesting, therefore, that pregnancy also seems to exert a beneficial influence on hyperthyroidism. In our own limited series of cases, treatment

has been discontinued either during or at the end of gestation, and in no instance has a relapse so far occurred.

It has been feared that antithyroid therapy might induce goiter or cretinism in the child. True, large doses of antithyroid drugs have influenced the thyroid of the animal fetus. But the situation is quite different in human beings that are being treated for hyperthyroidism. The dose is relatively much smaller, and the mother is maintained in a state of normal thyroid function or, at the most, slight hypothyroidism. The fetus presumably receives somewhat less antithyroid effect than the mother, and it seems unlikely that significant hypothyroidism would follow antithyroid therapy of the mother. In practice, the children of mothers born during or after treatment with antithyroid drugs are actually entirely normal (16). Only a few exceptions have been reported. One case, recorded by Eaton (15), was delivered by cæsarian section after the mother had received what may have been excessive dosages of thiouracil and had a goiter at the time of birth. Even under these unfavorable circumstances, the subsequent development of the infant was normal. Several authors have cautioned against the use of these compounds during pregnancy, but their reasons do not seem to be well substantiated

Menopause. It is a common belief that hyperthyroidism occurs more frequently at the time of the menopause. Its importance arises from the fact that it may be difficult to determine which symptoms are due to hyperthyroidism and which to the menopause. When antithyroid therapy is used, complete control of all objective manifestations of hyperthyroidism is frequently observed, while vague and ill-defined symptoms continue. These often improve or disappear entirely when estrogen is also given. Some have suggested that in patients with hyperthyroidism and the menopause treatment of the menopause may relieve the hyperthyroidism Certainly, it is common experience that women in menopause have frequently experienced lasting remissions from antithyroid therapy, and this seems to be especially true if symptomatic treatment with estrogen is also given This observation would fit with the claims that a certain percentage of women with hyperthyroidism are apparently cured by estrogen therapy alone. Sometimes typical menopausal symptoms are revealed for the first time when the coexisting hyperthyroidism is controlled

Diabetes There seems to be no very close connection between

hyperthyroidism and diabetes, though the two diseases may occur together. Cure of the hyperthyroidism is sometimes associated with an improvement or complete disappearance of diabetes, but in other instances the diabetes continues unchanged after the hyperthyroidism has been controlled. If hyperthyroidism develops after the diabetes has become established, there is no apparent reason for expecting the latter to be improved when the hyperthyroidism is controlled. On the other hand, if the diabetic state is revealed for the first time by the development of hyperthyroidism, an apparent cure may follow when the rate of metabolism is reduced to normal.

Nodular Goiter with Hyperthyroidism

There is a difference of opinion as to whether a distinction should be made between Graves' disease and the hyperthyroidism associated with nodular goiter. In their most classic forms, these two types of hyperthyroidism differ clearly. Graves' disease supposedly occurs in young people and is associated with a diffuse symmetric enlargement of the thyroid gland and with typical eye signs of varying severity. Hyperthyroidism with nodular goiter, sometimes referred to as toxic adenomatous goiter, is supposed to occur in older people and to be unassociated with eye signs; many of the other features of hyperthyroidism may also be lacking, according to some observers. Though one can easily make such a distinction in cases which follow this classic pattern, there are a great many cases which cannot with certainty be placed in one or the other group.

It is well recognized that eye signs may be completely lacking in cases which otherwise would be diagnosed as Graves' disease. On the other hand, the classic eye signs of Graves' disease can occur in older people with hyperthyroidism, and it is not unusual for the signs to be associated with a goiter which is not of the diffuse, regular variety, but with one which is unevenly enlarged, if not truly nodular.

Recent studies by Leblond *et al* (30) have shown that even in the so-called toxic adenomatous goiter the hyperactive tissue is not the nodular part of the thyroid but the intervening thyroid tissue There are rare cases, as Cope (11) has pointed out, where the hyperthyroidism seems to be due to an overactive thyroid nodule. However, it is common clinical observation that a nodular goiter may be present for many years prior to the onset of hyperthyroid-

ism, and it is only rarely that one observes hyperthyroidism to develop concomitantly with the appearance of an enlarging thyroid nodule The paranodular tissue, which seems to be the usual site of origin of the hyperthyroidism, would appear to differ in no essential anatomic respect from the diffuse hyperplasia classically associated with Graves' disease. For the most part, it is, therefore, impractical and possibly also unwise to regard these two clinical variants as two different diseases. Experience with antithyroid therapy has not aided in making a distinction between the two conditions. Indeed, the response to treatment may be identical in the two types of hyperthyroidism. The only notable exception to this is the delayed response exhibited by some cases with large nodular goiters.

Several observers have described long delays in cases with large nodular goiters, especially if iodine therapy has previously been given. It seems clear that the latent period after treatment is started, which precedes an improvement in the hyperthyroidism, is directly related to the amount of thyroid hormone stored within the gland. This is easily understandable in the case of the normal thyroid gland, for one might assume that nearly all of the normally stored hormone could be secreted before any evidence of hypothyroidism would ensue It is of interest, therefore, to speculate about what happens in the large nodular goiters. Histologically, these show all stages of thyroid hyperplasia and atrophy, and usually the larger part of the gland is made up of nodules which resemble colloid goiter. Often these atrophic areas are surrounded by dense fibrous tissue, and one might conclude that these areas are completely inactive and unresponsive However, in order to explain the delayed response to antithyroid therapy, it must be assumed that the large amount of hormone stored in the inactive portions of the gland can somehow be mobilized. This would imply that the so-called involutionary nodules are still capable of relatively normal function. Though this line of reasoning seems to lead to an unlikely conclusion, the observation that these nodules sometimes disappear under antithyroid therapy would support the view that the nodular goiter is still capable of responding to normal physiologic stimuli. Aside from the occasional long latent period, hyperthyroidism with nodular goiter behaves in much the same way as Graves' disease when antithyroid therapy is given

It is of interest to recall that many surgeons claim that Graves'

disease tends to relapse after surgery but that toxic adenomatous goiter is almost always cured by operation. It is further of interest to note that many surgeons have recommended the mere removal of nodules as a treatment for hyperthyroidism. It is truly remarkable that the *simple removal of one or more involutional nodules should* cure hyperthyroidism, if the latter condition is due to involvement of paranodular tissue only. However, the good results obtained by surgery in nodular goiter might indicate that the incidence of lasting remissions after antithyroid therapy might be greater in this condition than in classic Graves' disease. Published data on this point are not as yet adequate to answer this question

TABLE III

Percentage Incidence of Thyroid Nodules in 1,000 Autopsies (24)

Age, years	White		Negro	
	Male	Female	Male	Female
18–21	—	—	2	—
21–31	5 5	18 7	5 5	6 4
31–40	22 8	30	12 5	15 3
41–50	23	40 7	11 4	32
51–60	29.4	63 2	17 9	44
61–70	46	72	33	78.5
Above 70	44 1	80	25	50

Thyroid Carcinoma In recent years there has been an increased interest in malignancies of the thyroid gland There are only scant references to thyroid carcinoma in the older literature, but along with the increasing tendency to do prophylactic surgery of carcinoma in various parts of the body, an attempt has been made to define what type of thyroid lesion might constitute a precancerous state. The major conclusion which seems to have been reached is that thyroid cancer tends to arise in single thyroid nodules, and it has been suggested (22) that all nodular goiters should be subtotally resected as a means of preventing the development of thyroid cancer This problem requires further study because of the high incidence of nodular enlargement of the thyroid in the general population Jaffe (24) observed that in the Chicago area 32 to 80 per cent of women above 40 years of age examined postmortem had nodules in the thyroid gland. Schlesinger, Gargill, and Saxe (49) in

Boston found thyroid nodules, 1 cm. or more in diameter in 8.2 per cent of persons. On the other hand, death due to cancer of the thyroid is rare. VanderLaan (56) found that fewer than 19 deaths were due to this cause, after analyzing more than 35,668 autopsies. Rogers, Asper, and Williams (47) noted that of 544,918 patients admitted to large general hospitals only 64 were found to have malignant tumors of the thyroid gland, as determined histologically. The very high incidence of carcinoma found by some observers in thyroid nodules which have been removed surgically suggests that the criteria used in making this diagnosis need revision

TABLE IV

Incidence of Thyroid Carcinoma in Autopsy Material, as Reported from Several Sources

Author	*Locality*	*Number of autopsies*	*Thyroid carcinomas found*	*Deaths from thyroid carcinoma*
Ophuls (37)	San Francisco	3,000	0	0
Jaffe (24)	Chicago	1,000	2	2
Schlesinger *et al* (49)	Boston	2,185	5	0
VanderLaan (56)	Boston	>35,668	<20	<19

It has been suggested that thyroid malignancy might be induced if an antithyroid compound be given for long enough. It has been shown in rats and mice that malignant thyroid tumors do develop if the thyroid gland is kept in a state of hyperplasia for most of the animal's lifetime This tendency seems to be increased by the simultaneous administration of a carcinogenic substance The suggestion that patients with hyperthyroidism might behave in a similar fashion has not been supported by actual trial. It would seem unlikely that malignancy would be induced for two reasons. (*1*) small doses are given for a very limited fraction of the individual's lifetime; (*2*) the dosage employed tends, in the long run, to induce regression of the thyroid gland rather than increase its hyperplasia. Rienhoff's (43) observation that many years after hyperthyroidism has been cured by subtotal thyroidectomy the hyperplastic picture may still remain is reassuring. Yet cancer is a most unusual sequel to subtotal thyroidectomy for hyperthyroidism It is not yet known whether or not the thyroid gland remains hyperplastic when a lasting remission has been achieved by antithyroid therapy. Recent data ob-

tained by the use of radioactive iodine suggests that lasting remissions are associated with a reversion of the hyperplasia to normal. It would seem reasonable to conclude, therefore, that the possibility of thyroid carcinoma should not influence the decision as to the use of antithyroid treatment, and it would also seem justified not to alarm the patient, should a nodule be present in the thyroid gland.

References

1 Aranow, H, Elliott, R. H. E, Frantz, V. K, Melcher, G. W., and Werner, S C.: Ann. Surg *124*, 167, *1946*.
2 Astwood, E B: J Clin Endocrinol *5*, 345, 1945
3 Astwood, E B, and Stanley, M. M.: West. J. Surg. *55*, *625*, 1947.
4 Astwood, E B, and VanderLaan, W. P: J Clin. Endocrinol. *5*, 424, 1945 Ann. Int. Med *25*, 813, 1946
5 Barr, D. P, and Shorr, E: Ann. Int. Med *23*, *754*, *1945*.
6 Bartels, E C.: J A M. A *129*, 932, *1945*
7 Bartels, E C. Am. J. Med *2*, 150, 1947.
8 Beierwaltes, W H., and Sturgis, C C J A M A *131*, *735*, *1946*
9 Beierwaltes, W. H, and Sturgis, C C. J Lab & Clin Med. *32*, 392, 1947.
10 Berman, L Proc. Soc. Exper. Biol. & Med. *59*, 70, *1945*.
10a Christensen, H. N, and Cooper, P F: Unpublished data
11 Cope, O, Rawson, R W, and McArthur, J W Tr Am Assoc Study of Goiter *1946*, 156
12. Crile, G Canad M A J *57*, 359, *1947*
13 Cruzeoke, E, Musica, G R, and Diaz de Valdes, H: Res méd de Chile *73*, 223, 1945.
14 Danowski, T S, Man, E B, and Winkler, A W J Clin Investigation *25*, 597, 1946
15 Eaton, J C.: Lancet *1*, *171*, *1945*
16. Freiesleben, E, and Kjerulf-Jensen, K Clin Endocrinol 7, 47, 1947
17 Frisk, A. R. Nord Med *31*, 1575, 1946
18 Hadorn, W, and Beer, J Schweiz med Wchschr *76*, 1101, 1946
19. Hercus, C E New Zealand M J *45*, *321*, *1946*
20 Hercus, C E, and Purves, H D New Zealand M J *43*, *213*, *1944*
21 Himsworth, H P, Morgans, M E, and Trotter, W R Lancet *1*, 241, 1947
22 Hinton, J W, and Lord, J W J A M A *129*, 605, 1945
23. Holten, C Lancet *2*, *695*, *1946*
24. Jaffe, R. H: Arch Path *10*, *887*, *1930*
25. Kjerulf-Jensen, K, and Meulengracht, E Nord Med *32*, *2809*, *1946*
26. Leys, D Lancet, *1*, *461*, *1945*
27. McCullagh, E P, Ryan, E J, and Schneider, R Cleveland Clin Quart *13*, 232, 1946.

28 McGavack, T. H, Gerl, A. J, Vogel, M., and Schutzer, S : Am J. Med. *2*, 144, 1947.
29 Magnusson, P, and Sorensen, G. Acta med Scandinav. *125*, 263, 1946.
30 Leblond, C. P., Fertman, M B., Puppel, I D, and Curtis, G. M.: Arch. Path *41*, 510, 1946.
31. Means, J. H : Ann Int Med *23*, 779, 1945
32 Meulengracht, E, Kjerulf-Jensen, K., and Schmidt, K.: Lancet *2*, 207, 1946
33 Meulengracht, E, and Schmidt, K.. Acta med. Scandinav. *122*, 294, 1945.
34 Miller, W. H, Roblin, R O, and Astwood, E B.· J Am. Chem. Soc. 67, 2201, 1945.
35 Moore, F. D.· J A M. A *130*, 315, 1946
36 Morgans, M E · Lancet *1*, 519, 1947
37 Ophuls, W · Stanford Univ Publ, Univ Series, M. Sc *1*, 131, 1926.
38 Palmer, M V Ann. Int. Med *22*, 335, 1945
39 Perrault, M, and Bovet, D. Lancet *1*, 731, 1946
40 Poate, H R. G M. J. Australia *2*, 93, 1947.
41 Poate, H R G., and Spencer, S L : M J Australia *1*, 493, 1946
42 Rawson, R W, Evans, R D, Means, J H, Peacock, W C., Lerman, J., and Cortell, R E : J. Clin Endocrinol *4*, 1, 1944
43 Rienhoff, W F Arch Surg *13*, 391, 1926
44 Reveno, W. S Ann Int Med. *25*, 822, 1946.
45. Reveno, W S J A M A *133*, 1190, 1947
46 Ritchie, F L, and Geddes, B L · M J Australia *1*, 381, 1944.
47 Rogers, W F, Asper, S P, and Williams, R H. New England J Med *237*, 569, 1947
48 Rose, E, and McConnell, J · Am. J M. Sc *213*, 74, 1947.
49 Schlesinger, M S, Gargill, S. L, and Saxe, I H . J. A M A. *110*, 1638, 1938
50. Schachner, H, Franklin, A L, and Chaikoff, I. L.: Endocrinology *34*, 159, 1944
51 Spuhler, O · Schweiz med Wchnschr 77, 275, 1947
52 Stanley, M. M, and Astwood, E B : To be published
53 Stanley, M M., and Astwood, E B Endocrinology *41*, 66, 1947
54 Stanley, M M., and Astwood, E B Endocrinology *42*, 107, 1948
55 Thyssen, J. Acta med Scandinav *121*, 422, 1945
56 VanderLaan, W P . New England J. Med *237*, 221, 1947.
57. VanderLaan, W. P, and Bissell, A Endocrinology *39*, 157, 1946.
58 VanderLaan, J E, and VanderLaan, W. P · Endocrinology *40*, 403, 1947.
59 Van Winkle, W., Hardy, S M, Hazel, G R., Hines, D C., Newcomer, H S, Sharp, E A, and Sisk, W. N J A M. A *130*, 343, 1946
60 Williams, R H J. Clin Endocrinol *5*, 210, 1945.
61 Williams, R H J Clin Endocrinol. *6*, 1, 1946.
62 Williams, R. H . Arch. Int Med *80*, 11, 1947.
63 Wilson, A. Lancet *1*, 640, 1946

64 Wilson, A, and Goodwin, J Lancet *1*, 669, 1947
65 Wilson, D B: Ann Surg *74*, 129, 1921
66 Winkler, A W, Man, E B, and Danowski, T. S: J. Clin Investigation *26*, 446, 1947
67. Wolff, J, Chaikoff, I L, Taurog, A, and Rubin, L: Endocrinology *39*, 140, 1946

Diagnosis of Disease by Enzymic Methods*

CHARLES HUGGINS AND PAUL TALALAY
The University of Chicago, Chicago, Illinois

This paper discusses what may be learned from the enzymes of blood serum about the kinds and amounts of diseases and unusual tissue activities in the body. Certainly the greatest handicap to progress in contemporary clinical medicine, and this is particularly pointed in the cancer problem, is the lack of simple and precise technics for investigating abnormal states in the intact organism It is self-evident that a deficiency of quantitative methods reflects crudeness in a science By their intrinsic nature enzymes lend themselves to technics, often simple and precise, which demonstrate many subtle and otherwise inscrutable abnormalities—frequently in elegant ways.

Enzymes have been found in all living cells and doubtless are essential to life All of the enzymes which have been crystallized were found to be proteins of high molecular weight which differ from other proteins in having catalytic activity In order to function, the living cell carries out many specific chemical reactions that do not take place when the reactants are simply mixed together The enzymes have the remarkable property of effecting considerable syntheses or degradations of matter in the bland environment of the cell with its cool temperature, these same reactions in the test tube frequently require great activating forces, such as strong chemicals, high temperatures, or great pressures The enzymes are catalysts, and by definition they are not consumed in the reactions which they promote and theoretically are capable of an infinite amount of work

* Some of the studies described in this paper were supported by grants from Mr Ben May, Mobile, Alabama, from the Committee for Research in Problems of Sex of the National Research Council, and from the American Cancer Society recommended by the Committee on Growth, the National Research Council

Michaelis and Menten (61) proposed the hypothesis that an enzyme unites with its substrate to form a chemical intermediate, and this view has been adopted by the majority of enzymologists as best explaining the known facts. The Van Slyke (78) concept of enzyme-substrate action, while obviously crude, provides a useful working model. It is now known that chemical reactions need some energy of activation before they can proceed. A catalyst apparently acts by lowering the activation energy of the reaction, the velocity of which is proportional to the number of activated molecules present (74).

The work of Beadle and Tatum (6) on the bread mold (*Neurospora crassa*) has demonstrated that the genes control or regulate specific reactions in a system either by acting directly as enzymes or by determining the specificities of enzymes. Spiegelman and the Lindegrens (73), in their studies of what they have called the adaptive enzymes, showed that the gene may initiate the synthesis of these enzymes in the presence of a specific substrate. One of their experiments (73) may be briefly summarized. The strain of yeast Saccharomyces carlsbergensis and all its haploid offspring can adapt to ferment the carbohydrate melibiose while the strain Saccharomyces cerevisiae and its progeny are unable to do so. The progenies of hybrids between these strains were cultivated in the presence and absence of melibiose. In the absence of this sugar, a 2.2 ratio of fermentors to nonfermentors was exhibited by the four haploid segregants from the hybrid However, in the presence of melibiose all four hybrids and the clones derived from them possess the power of melibiose fermentation, and they all maintain it indefinitely provided they are kept in contact with this sugar; but when the melibiose is removed, only two of the four hybrids can readapt to its fermentation. The transfer of this newly adapted character from one cell generation to the next is apparently effected by the enzyme molecules contained in the cytoplasm.

For some years the similarities between the antigen-antibody and the enzyme-substrate relationships have been apparent (52), and in both cases specificity is related to steric configuration. Antibodies produced by the injection of crystalline enzymes inhibit to some degree the reaction of an enzyme with its substrate.

Pauling (65) has elaborated an interesting hypothesis about the

nature of enzyme action: An enzyme has a structure closely similar to an antibody, except that the surface configuration of the enzyme is not so closely complementary to its specific substrate as is that of the antibody to its homologous antigen. An enzyme is closely complementary to its specific substrate, as the antibody is to its homologous antigen. However, the enzyme is closely complementary to an unstable molecule which has only a transient existence, the "activated complex."In the Pauling theory, the enzyme has a small power of attraction to the substrate molecule that is sufficient, however, for an attachment to occur on its active surface regions. The attached substrate molecule is strained by the forces of attraction of the enzyme which thereby deform it into the configuration of the activated complex for which the power of attraction by the enzyme is greatest. The activated complex then undergoes change under the influence of ordinary thermal agitation.

Enzymes are active over a narrow range of temperature and have optimal activity at a characteristic hydrogen ion concentration which varies over the great range of pH 1.5 for pepsin (63) to pH 9.5 to 9.9 for arginase (43). Under set thermodynamic circumstances, enzyme action is reversible.

Many enzymes are influenced by specific activators and inhibitors. The activators range from such simple substances as magnesium ions for alkaline phosphatase (18,19) to the complicated coenzymes such as diphosphopyridine nucleotide for the dehydrogenases, as Harden and Young first showed for alcoholic fermentation. Inhibitors are particularly spectacular in the case of cholinesterase, and a number of inhibitor agents have been found which act in very small concentration; for example, physostigmine in one part per million (10^{-6} molar) concentration causes complete inhibition of this enzyme An interesting new inhibitor is synthetic pteroylglutamate, which strongly inhibits xanthopterin oxidase and xanthine oxidase in small amounts (44). Enzyme action, therefore, must be interpreted in relation to the amount of enzyme, the substrate concentration, the activators and the inhibitors present, as well as the time, temperature, and pH conditions.

The enzymes of tissues differ quantitatively, constituting the characteristic catalytic mosaic of each tissue; although in certain tissues an enzyme may be absent while in other tissues it has been

accumulated in large amount, each of the enzymes present apparently behaves qualitatively much the same in all tissues (27).

It has become apparent that some, but evidently not all, of the normal organs contribute proteins to the plasma pool. Frequently the contribution is small. The errors inherent in most of the protein methods forbid enzyme recognition by classical protein technics, but in studying the enzymes products of the organs comprising the body may be recognized This is so because the enzymic activity is cumulative with time, and the work of these catalytic bodies betrays their presence.

Eight enzymes have been discovered in the serum which throw light on many abnormal cellular processes in about twelve different tissues. In the following discussion of the enzymes that are of diagnostic usefulness, the high value of colorimetric enzyme assay technics will be apparent They frequently combine accuracy, simplicity, and delicacy

Amylase

The principal methods are based either on the amyloclastic action of amylase, as measured by the starch blue color with potassium triiodide (80) or by a decrease of viscosity of a starch paste (14,17), or by measuring the saccharogenic power of the enzyme, one of the most satisfactory methods being that of Somogyi (71)

In dogs, pancreatectomy causes a rapid fall of diastatic activity of the blood to one-half or one-third (12,51,57,66) When the diabetes is controlled with insulin, it has been reported that the enzyme may attain a normal value after several months Using Russell's (68a) colorimetric method, however, it was found that the amylase content of serum was decreased for many months, at least Subtotal pancreatectomy (51) evoked no amylase changes Cope and co-workers report that hypophysectomy (10) is followed by an abrupt rise within 2 days after operation, and that in the adrenal insufficiency following adrenalectomy it is even higher (11) McCaughan (57) found that ligation of the pancreatic ducts and inflammatory changes of the pancreas in the dog caused a brief rise of amylase (8–15 days), he emphasized the transitory character of abnormal blood concentration.

Since the original observation of Wohlgemuth (81), all workers are agreed that high values are obtained in inflammatory or obstructive pancreatitis in man. Elman (16) was able to make the diagnosis of acute pancreatitis in 65 cases in 5 years in a single hospital Somogyi (72) found that 33.4 per cent of 382 cases of diabetes have low serum amylase, as compared with that of 4.1 per cent of apparently normal subjects. McCall and Reinhold (56) found low serum amylase in portal cirrhosis and, usually, in gallbladder disease.

Applebaum (2) noted a sharp increase of serum amylase in epidemic parotitis, which was considerably higher in bilateral involvement than in unilateral disease.

The serum amylase is high in pancreatic and salivary gland inflammations and obstructions and is low in liver disease and pancreatic insufficiency High values have been reported following the removal of the adrenals or the pituitary of dogs.

Zymohexase

This enzyme catalyzes the splitting of fructose 1,6-diphosphate into two molecules of triose phosphate, and holds a key position in glycolysis The enzyme probably occurs in all cells, although its chief concentration is in the skeletal musculature. Warburg and Christian (79) demonstrated that the level of zymohexase in the serum of sarcoma-bearing rats is elevated roughly in proportion to the size of the tumor; increased values are not observed in pregnant rats

The method of Warburg and Christian is rather elaborate, depending on the reduction of diphosphopyridine nucleotide A simple and accurate colorimetric assay has been developed in our laboratory by Sibley and Lehninger (70). A significant rise of zymohexase occurred in rats inoculated with sarcoma 39 and Walker tumor 256 Elevations of serum zymohexase comparable to those of tumor-bearing rats are not often seen in patients with cancer, apparently because the ratio of tumor to host is much greater in the rat. However, *moderate elevations of zymohexase occur in many cases of human cancer and high elevations in a few* Increases of serum zymohexase have also been observed in progressive muscular dystrophy.

Histaminase and Diamine Oxidase

In 1929, C. H. Best found that, in the presence of oxygen, histamine was destroyed by extracts of kidney and intestine. While Best believed that histaminase was specific, E. A. Zeller found that it attacks practically all diamines. Holmberg and Laurell (36a) have recently shown that the copper-containing blue serum globulin *is a component of the histaminolytic enzyme system of plasma.* Ahlmark (1) measured the histaminase content of plasma by a method of bio-assay of residual histamine, utilizing the contractile power of the guinea pig intestine as an index. The plasma of healthy men and women contains small amounts of this enzyme, but there is a progressive increase in pregnancy in which a thousandfold increment often occurs. This bio-assay method is cumbersome.

Zeller and Birkhauser (83) measured the splitting of the diamine, cadaverine, gasometrically by dialyzed serum. Findings similar to those of Ahlmark were obtained; the tests require many hours for their performance. *Elevation of diamine oxidase (histaminase) occurs during pregnancy.*

Alkaline Phosphatase

The phosphatases, several in number, are enzymes which dephosphorylate phosphoric esters. One of them, alkaline phosphatase, attacks monophosphoric esters; its optimum *p*H activity is around *p*H 9 3 Susuki and co-workers (75), in 1907, employed inositol hexaphosphate as a substrate, but the hydrolysis was very slow One of the earliest demonstrations of the activity of this enzyme was by Levene and Medigreceanu (50), who showed that intestinal juices decomposed nucleotides and especially guanylic acid liberating phosphate groups. The following year, Grosser and Husler (29) found that extracts of intestine and kidney hydrolyzed glycerophosphate. Robison (68) discovered that this enzyme was particularly abundant in growing bone and cartilage, and that its activity was greatest between *p*H 9 and 9 5 Kay (45) made the important discovery that the alkaline phosphatase in the serum is increased in states of osteoblastic proliferation, such as in growing children, in osteitis fibrosa (hyperparathyroidism), in osteitis deformans, in rickets, and locally around metastases of neoplasms to bone. Later

work showed that the plasma values were particularly high in carcinoma of the prostate which had metastasized to bone, a tissue wherein this tumor flourishes and usually evokes an osteoblastic response. It is also increased in osteogenic sarcoma. Franseen and McLean (22) were able to follow the unfavorable course of an osteogenic sarcoma by observing the alkaline phosphatase content of the serum, and we have confirmed this observation. Woodard and Craver (82) found that alkaline phosphatase is frequently elevated in Hodgkin's disease, and concluded that bone changes probably occur more often in this disease than the incidence of overt lesions would indicate. *The alkaline phosphatase of serum reflects osteoblastic activity.*

Umeno (77) and others (3,23) found that bile contains rather large amounts of alkaline phosphatase. Roberts (67) discovered that in obstructive jaundice in man, the alkaline phosphatase is increased, while in hemolytic icterus it is normal. This important work has been confirmed and extended. The Gutmans and coworkers (34) established that the alkaline phosphatase of serum was elevated in most cases of obstructive jaundice, liver metastasis, and hepatitis resulting from hepatotoxic drugs (e g , arsenotherapy). The enzyme is usually at normal levels in hemolytic jaundice, cirrhosis of the liver, and "catarrhal" jaundice. These workers (34) state: "In general the determination of serum phosphatase activity affords evidence of limited but definite value in the major practical problem presented by patients with jaundice: the decision between surgical intervention ('surgical jaundice') and conservative management ('medical jaundice')." We have found repeated determinations of alkaline phosphatase to be of great value in the differential diagnosis between functional pain and residual stones in the biliary tract after choledocholithotomy (Fig. 1).

The effects of experimental biliary obstruction on the alkaline phosphatase content of the serum differ in the cat and the dog. In the cat (21), ligation of the common duct is not followed by a striking rise of alkaline phosphatase, but its urinary content rises markedly. In the dog (3), common duct ligation causes a prompt and marked elevation that soon reaches a plateau which persists for many months In our observations, a nearly equal elevation in the serum is achieved and is maintained in all dogs, that is, a level between 90 and 120 King-Armstrong units per hundred cubic cen-

timeters Relief of the obstruction causes a decrease of phosphatase at different rates; either a prompt and marked fall in early obstruction, or slow decreases associated with liver damage (Fig 2). Following pancreatectomy in the dog, there is a slight and progressive rise in alkaline phosphatase, reaching high values when fatty liver

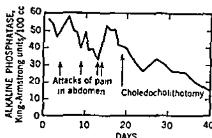


Fig 1. Alkaline phosphatase of serum in patient with stones in the common bile duct The patient had had calculi removed from the common duct 3 weeks previous to this study Following operation his jaundice disappeared but the patient continued to have attacks of epigastric distress, at first these colicky pains were attributed to a functional bowel disturbance, but since each attack was followed by a rise in alkaline phosphatase of the serum, the common bile duct was re-explored and a stone removed with cure.

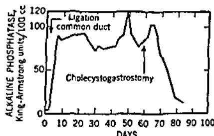


Fig 2 Rise of alkaline phosphatase following ligation of the common bile duct in a dog During the obstruction, an occult worsening of liver disease occurred between day 42 and 50, judged from the rise of phosphatase in the serum Cholecystogastrostomy was followed by a temporary increase of alkaline phosphatase for 6 days, then a return to normal

has developed It now seems proved that the rise in liver disease is to a large extent due to absorption into the serum of alkaline phosphatase fabricated by the liver, rather than to a failure of excretion by the liver of phosphatase produced elsewhere, obstructions of a biliary duct draining a small part of the liver cause a rise of alkaline phosphatase (24,33), and in the rat hepatomas contain large amounts of alkaline phosphatase (28) *Alkaline phosphatase of serum reflects obstruction of biliary ducts and damage to the liver cells.*

We have found (42a) that alkaline phosphatase can enter the

blood stream from the distended intestine of dogs High intestinal obstruction, produced by transection of the jejunum (Fig. 3) with suture of the inverted ends, causes only moderate elevation of alkaline phosphatase, whereas the same intestinal obstruction at the terminal ileum (Fig 4) produces much higher values A closed loop of jejunum produces much higher values (Fig. 5) than a closed loop of ileum The elevated values with closed intestinal loops are unsustained; clearly, absorption from a distended bowel is much greater in the early phases of distention than in later stages In the interpretation of the results, it should be borne in mind that the alkaline phosphatase content of the jejunum (about 60 King-Armstrong units per gram) is considerably higher than that of the

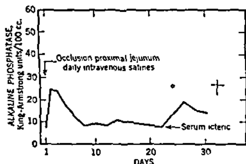


Fig 3 Alkaline phosphatase in serum of a dog with induced high intestinal obstruction Note the terminal involvement of the liver as reflected in the changed levels of the enzyme

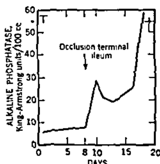


Fig. 4 Alkaline phosphatase in serum of a dog subjected to low intestinal obstruction.

ileum (about 15 units per gram). In jejunal obstruction, loss by vomiting of the intestinal juices rich in alkaline phosphatase occurs promptly, with only slight increase of serum levels; while a low intestinal obstruction causes great dilatation of the whole intestine above it, with absorption of phosphatase therefrom. In closed jejunal loops, with the resulting tremendous distention with fluid concentrated in alkaline phosphatase, absorption effects higher serum phosphatase values than comparable distention of the ileum with low phosphatase-containing fluid *Elevation of alkaline phosphatase of serum can result from absorption from distended intes-*

tine; the rises result from much more drastic changes than are required for increases resulting from liver or bone disturbances.

Acid Phosphatase

A phosphatase with an activity maximum around pH 5 was discovered in 1934 in extracts of the spleen and kidney of swine and cattle (4,13). This enzyme, acid phosphatase, was found by Kutscher and Wolbergs (49) in rich concentration in the human prostatic gland. Some very ingenious observations were then made

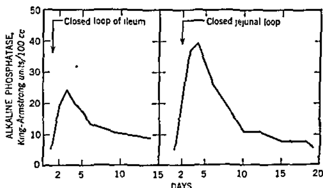


Fig 5 Alkaline phosphatase in serum of dogs with a closed loop of ileum and jejunum, respectively. A 6 inch segment of the intestine was prepared as a closed loop by inverting the ends according to the method of Dragstedt, continuity of the intestine being re-established. Note the temporary character of the rise of alkaline phosphatase

by A B. and E. B. Gutman. In infancy and childhood the enzyme is present in the prostate in small amounts, during puberty the amounts gradually increase (30) to reach the high values found in adults, representing a secondary sex characteristic of a chemical type. The low values of the prostate of immature monkeys became markedly increased (31) after injection of androgen, but not of estrogen Gutman, Sproul, and Gutman (35) found that acid phosphatase is markedly increased at the site of prostatic metastasis in bone. In 1938, both Gutman and Gutman (32) and Barringer and Woodard (5) reported that acid phosphatase of serum was frequently increased in patients with metastatic cancer of the prostate.

In a series of 47 patients (38) with advanced prostatic cancer, 21 had significant elevation of acid phosphatase *Significant elevation of acid phosphatase indicates with certainty the diagnosis of metastatic cancer of the prostate.*

Determination of both acid and alkaline phosphatases is essential in both the diagnosis and the treatment of prostatic cancer Frequently, the diagnosis is completely in doubt until these enzymes have been measured. A high acid phosphatase value with a normal alkaline phosphatase usually signifies metastasis to lymph nodes; an accompanying elevation of alkaline phosphatase indicates significant involvement of bone or liver. Herbert (36) reported that prostatic acid phosphatase is inactivated by incubation of the serum for one hour at 37 C. or by treatment of the serum with $^2/_5$ volume of ethyl alcohol for one-half hour at room temperature; she recommends the latter method to differentiate prostatic from nonprostatic acid phosphatase

The hormonal treatment of prostatic carcinoma, which originated in this laboratory (38,41), was proved to be effective by a study of the phosphatases. The whole panorama of metastatic cancerous activity may at times be demonstrated mathematically by enzymic determination at frequent intervals.

Chromogenic Substrates

This technical approach has simplified many enzymic determinations. A chromogenic substrate is a compound in which the chromophore group of an acid-base indicator is replaced with a known hydrolyzable group to produce a colorless substrate which on enzymic action yields color, the measurement of the amount of color produced indicates the amount of enzyme present.

The technic was first used by Derrien (15) in a qualitative way; he discovered an extract of a certain shellfish, Murex trunculus, which contained phenolsulfatase The latter enzyme hydrolyzed indoxyl sulfates obtained from human urine with the liberation of indigo in a blue form, the royal purple of the ancients. Ohmori (64) and others (7,37) employed a phosphate ester of *p*-nitrophenol as a substrate for phosphatase determinations, measuring the free *p*-nitrophenol liberated colorimetrically King (47) synthesized the calcium and barium salts of various phthalein phosphates, including phenophthalein, but found that their hydrolysis was too slow to be

a convenient substrate; however, Bray and King (8) used these esters as a semiquantitative measure of alkaline phosphatase liberated during bacterial growth

We synthesized a freely soluble ester, sodium phenolphthalein phosphate, which proved to be convenient for use in phosphatase tests (42). Chromogenic substrates have also been synthesized in our laboratory for the determination of sulfatase (40), esterase (39), and glucuronidase (76).

β-Glucuronidase

Masamune (53) first defined the enzyme that splits the glucuronide linkage as "glucuronidase", in the early work, menthol, phenol and borneol esters of glucuronic acids were used. We achieved the biosynthesis of phenolphthalein glucuronide by injecting rabbits intramuscularly with sodium phenolphthalein phosphate which was hydrolyzed by phosphatase, conjugated with glucuronic acid, and excreted as the glucuronide in the urine, from whence it was extracted (76); our simple method of performing glucuronidase assays could then be devised McDonald and Odell (58) and Fishman (20), using this method, found that the glucuronidase activity of plasma increases progressively during pregnancy and drops to normal levels after parturition During pre-eclampsia and eclampsia, striking elevations of glucuronidase occur as compared with normal gravidity and hypertensive toxemia (58). *In pregnancy, β-glucuronidase is increased and the values are particulary high in eclamptic toxemias*

Esterases and Lipase

These enzymes catalyze the hydrolysis of short-chain and long-chain fatty acids Most workers are now in agreement that there exist two types of esterases, one of the esterases splits acetylcholine preferentially, while the other esterase is much less specific The latter has been studied by a wide variety of technics, in which the substrates most commonly used have been ethyl butyrate and tributyrin Lipase is a designation used by many workers to characterize an esterase which acts preferentially on esters of long-chain fatty acids, such as olive oil

The work of Mendel and Rudney (60) in the esterase field has

been ingenious and fruitful According to their analysis of the problem, there exist in the animal body two esterases which are capable of splitting acetylcholine One of these enzymes, true cholinesterase, acts only on certain choline esters and is especially abundant in brain tissue and erythrocytes; the other esterase, pseudo cholinesterase, hydrolyzes not only esters of choline but a variety of nonspecific esters as well, and is found in horse serum and dog pancreas, among others Acetylcholine is split by both esterases (59); acetyl-β-methylcholine is hydrolyzed by true cholinesterase but not by pseudo cholinesterase, benzoylcholine is hydrolyzed by pseudo cholinesterase but not by true cholinesterase They find that the predominant esterase of human plasma is pseudo cholinesterase. According to their theory, neither acetyl-β-methylcholine nor benzoylcholine is hydrolyzed by the "common esterase," an enzyme capable of hydrolyzing glycerides and aliphatic esters. The stimulating work of Mendel and Rudney suffers from an ineptness of terminology. for example, what is "pseudo choline" or a "pseudo enzyme"? Our findings indicate that their concept is unnecessarily complex.

In this discussion we shall assume that there exist in the body three esterases acting on esters of organic acids· cholinesterase, nonspecific esterase, and lipase

A simple colorimetric technique has been devised, employing several esters of *p*-nitrophenol with fatty acids as chromogenic substrates (39). These substrates are preferentially split by nonspecific esterase of serum, and typical substrate patterns, which depend somewhat on species and *p*H, emerge

Khanolkar and Chitre (46) reported that esterase (ethyl butyrate) was higher in cancer-susceptible mice (strains A and C_3H) than in cancer-resistant strains (C_{57} black), but this has not been confirmed (69). Green (25) found that during the growth of Jensen sarcoma in rats the esterase content of the serum falls progressively to a very low level and that the esterase content of liver, lung, and kidney is much diminished; these observations have been extended for other transplantable rat tumors (62). Clearly, the presence of a tumor in a distant site produces liver damage which includes an impairment in the capacity of the liver to synthesize simple esterase With the exception of hepatoma (28), tumors possess very

little esterase; even neoplasms of the liver had less esterase (26) than normal liver.

McArdle (54) showed that 79 per cent of 71 patients with liver disease had abnormally low esterase values and that improvement or impairment of liver function was accompanied by a rise or fall, respectively, of the enzyme that hydrolyzes acetylcholine. Kunkel and Ward (48) found that determination of esterase (acetylcholine) in plasma was of value in differentiating hypoproteinemic states; in patients who had low plasma albumin, those with infectious hepatitis or cirrhosis had defective formation of esterase while those with the nephrotic syndrome showed a normal or hypernormal formation of this enzyme. German civilian internees on an inadequate ration had lower serum cholinesterase values than those who were full fed (55); it is inferred that the average level of pseudo cholinesterase in the serum is a delicate index of the state of nutrition of a population. *Low serum esterase is related to decreased function of the liver.*

The serum of normal dogs has slight or no activity in hydrolyzing olive oil (9). Following pancreatic duct ligation in the experiments of Cherry and Crandall (9), a lipase appeared in the blood, the maximum values being reached 24 hours after operation, at which time changes in esterase were very slight; the elevation was maintained for several weeks. McCall and Reinhold (56) found that the lipase in the serum increased in 12 of 13 patients with pancreatitis and in 9 of 16 patients with pancreatic cancer. In their opinion, high lipase and normal amylase values in the presence of painless jaundice suggest cancer of the pancreas *An increase of serum lipase indicates pancreatic disease.*

Discussion

Changes in the serum content of eight enzymes have been found to reflect disease of the following tissues. liver, excretory apparatus of the pancreas, salivary gland, muscle, osteoblasts, small intestine, and prostatic gland. It is not certain why elevations of zymohexase occur in certain cases of malignancy; since the content of this enzyme in tumors is greatly reduced, the tumor is not the source of the increase. Likewise, it is not known why β-glucuronidase or diamine oxidase increases in pregnancy.

TABLE I

Changes in the Enzymes in Serum in Disease

Enzyme	Organ involved	Enzymic change	Abnormal state
Amylase	Pancreas	Decrease	Pancreatectomy
	Liver?	Decrease	Diabetes mellitus
	Liver	Decrease	Hepatic cell damage
	?	*Increase*	*Hypophysectomy*
	?	Increase	Adrenal insufficiency
	Pancreas	Increase	Pancreatic duct obstruction and inflammation
	Salivary gland	Increase	Parotitis
Zymohexase.. ...	Muscle	Increase	Muscular dystrophy
	?	Increase	Cancer
Diamine oxidase	Placenta?	Increase	Pregnancy
Alkaline phosphatase....	Osteoblasts	Increase	Osteogenic sarcoma
	Osteoblasts	Increase	Rapid growth in children
	Osteoblasts	Increase	Rickets
	Osteoblasts	Increase	Metastasis to bone
	Osteoblasts	Increase	Hyperparathyroidism, etc
	Liver	Increase	Duct obstruction
	Liver	Increase	*Hepatic cell damage*
	Intestine	Increase	Obstruction
Acid phosphatase	Prostatic gland	Increase	Metastasis to certain organs
β-Glucuronidase	Decidua?	Increase	Pregnancy
	Liver?	Increase	Toxemias of pregnancy
Esterase.... .	Liver	Decrease	Hepatic cell damage
	Liver	Decrease	Malnutrition
Lipase	Pancreas	Increase	Duct obstruction

Enzyme deviations occur in an impressive list of over twenty-five disease conditions (Table I). One may confidently expect that, as more is learned about enzymes and about disease, this list will be extended.

Conclusion

The quantitative determination of enzymes in serum is extremely useful in the diagnosis of disease, and precision is often combined with elegance in the determination of subtle deviations from the quiescent state of activity in the normal.

References

1 Ahlmark, A Studies on the histaminolytic power of plasma with special reference to pregnancy Acta physiol. Scandinav. *9*, Suppl 28, 1944
2 Applebaum, I L Serum amylase in mumps Ann Int Med *21*, 35, 1944
3 Armstrong, A R, and King, E J. Phosphatase in obstructive jaundice Canad M A J *31*, 14, 1934
4 Bamann, E, and Riedel, E Ueber das Vorkommen zweir durch das pH Wirkungsoptimum unterscheidbaren Phosphoesterasen in tierischen Organen Ztschr f physiol Chem *229*, 125, 1934
5 Barringer, B. S, and Woodard, H Q Prostatic carcinoma with extensive intraprostatic calcification Tr. Am A Genito-Urin Surgeons *31*, 363, 1938.
6 Beadle, G W, and Tatum, E L Genetic control of biochemical reactions in neurospora Proc Nat Acad Sci *27*, 499, 1941
7 Bessey, O A, Lowry, O H., and Brock, M J A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J Biol Chem *164*, 321, 1946
8 Bray, J, and King, E J The phosphatase reaction as an aid to the identification of micro-organisms using phenolphthalein phosphate as substrate J Path & Bact *55*, 315, 1943
9 Cherry, I S, and Crandall, L A The specificity of pancreatic lipase Its appearance in the blood after pancreatic injury Am J Physiol *100*, 266, 1932
10 Cope, O, Hagerstromer, A, and Blatt, H The activity of the blood serum amylase in the hypophysectomized dog Am J. Physiol *122*, 428, 1938
11 Cope, O, Kapnick, I, Lambert, A, Pratt, T D, and Verlot, M G Changes in activity of blood serum amylase in response to changes in adrenal cortical function in the dog and rabbit Endocrinology *25*, 236, 1939
12 Cope, O, Kapnick, I, Lambert, A, Pratt, T D, and Verlot, M G Further observations of blood serum amylase activity in relation to pituitary, pancreas and thyroid function in the dog and rabbit Endocrinology *25*, 248, 1939
13 Davies, D R Phosphatase activity of spleen extracts Biochem J *28*, 529, 1934
14 Davison, W C A viscometric method for the quantitative determination of amylase Johns Hopkins Hosp Bull *37*, 281, 1925
15 Derrien Bull soc. chim biol *9*, 110, 1911
16 Elman, R Surgical aspects of acute pancreatitis J A M A *118*, 1265, 1942
17 Elman, R, and McCaughan, J M The quantitative determination of blood amylase with the viscosimeter Arch Int Med *40*, 58, 1927
18 Erdtman, H Glycerophosphatspaltung durch Nierenphosphatase und ihre Aktivierung Ztschr f physiol Chem *172*, 182, 1927.

19 Erdtman, H : Ueber Nierenphosphatase und ihre Aktivierung Ztschr. f physiol Chem *177*, 211, 1928.

20 Fishman, W. H β-Glucuronidase activity of the blood and tissues of obstetrical and surgical patients Science *105*, 646, 1947.

21 Flood, C A, Gutman, E B, and Gutman, A. B.· Serum and urine phosphatase activity in the cat after ligation of the common bile duct. Am J Physiol *120*, 696, 1937

22. Franseen, C C, and McLean, R . The phosphatase activity of tissues and plasma in tumors of bone. Am J Cancer *24*, 299, 1935

23 Freeman, S., and Chen, Y P.· The effect of jaundiced blood upon normal dogs, with special reference to the serum phosphatase. J Biol. Chem *123*, 239, 1938

24 Freeman, S, Chen, Y P, and Ivy, A C : On the cause of the elevation of serum phosphatase in jaundice J Biol Chem *124*, 79, 1938.

25 Green, H N Changes in the esterase and fat content of the serum induced by cancer and cancer-producing agents Brit J Exper Path *15*, 1, 1934

26 Greenstein, J P Esterase (butyric esterase) activity of normal and neoplastic tissues of the mouse J Nat Cancer Inst *5*, *31*, *1944*

27 Greenstein, J. P Biochemistry of Cancer New York, Academic Press, 1947

28 Greenstein, J P, and Leuthardt, F M Enzymatic activity in primary and transplanted rat hepatomas J Nat Cancer Inst *6*, 211, 1945

29 Grosser, P, and Husler, J Ueber das Vorkommen einer Glycerophosphatase in tierischen Organen Biochem Ztschr *39*, 1, 1912

30 Gutman, A B, and Gutman, E B "Acid" phosphatase and functional activity of the prostate (man) and preputial glands (rat) Proc Soc Exper Biol & Med *39*, 529, 1938

31 Gutman, A B, and Gutman, E B Adult phosphatase levels in prepubertal rhesus prostate tissue after testosterone propionate Proc Soc Exper Biol & Med *41*, 277, 1939

32 Gutman, A B, and Gutman, E B An "acid" phosphatase occurring in the serum of patients with metastasizing carcinoma of the prostatic gland J Clin Investigation *17*, 473, 1938

33 Gutman, A B, Hogg, B M, and Olson, K B Increased serum phosphatase activity without hyperbilirubinemia after ligation of hepatic ducts in dogs Proc Soc Exper Biol & Med *44*, 613, 1940

34 Gutman, A B, Olson, K B, Gutman, E B, and Flood, C A . Effect of disease of the liver and biliary tract upon the phosphatase activity of the serum J Clin Investigation *19*, 129, 1940

35 Gutman, E B, Sproul, E E, and Gutman, A B Significance of increased phosphatase activity in the site of osteoblastic metastases secondary to carcinoma of the prostate gland Am J Cancer *28*, 485, 1936

36 Herbert, F K · The estimation of prostatic phosphatase in serum and its use in the diagnosis of prostatic carcinoma Quart J Med *15*, 221, 1946

36a Holmberg, C G, and Laurell, C B Histaminolytic activity of a copper protein in serum Nature *161*, 236, 1948

37. Hudson, P B, Brendler, H, and Scott, W. W.: A simple method for the determination of serum acid phosphatase. J Urol *58*, 89, 1947.

38 Huggins, C, and Hodges, C V · The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate Cancer Research *1*, 293, 1941.

39 Huggins, C, and Lapides, J: Chromogenic substrates IV. Acyl esters of p-nitrophenol as substrates for the colorimetric determination of esterase J. Biol Chem. *170*, 467, 1947.

40 Huggins, C, and Smith, D R Chromogenic substrates III p-nitrophenyl sulfate as a substrate for the assay of phenolsulfatase activity J. Biol Chem. *170*, 391, 1947

41 Huggins, C, Stevens, R E, Jr, and Hodges, C V. The effects of castration on advanced carcinoma of the prostate gland Arch Surg *43*, 209, 1941

42 Huggins, C, and Talalay, P Sodium phenolphthalein phosphate as a substrate for phosphatase tests J Biol Chem *159*, 399, 1945

42a Huggins, C, and Talalay, P Unpublished data

43 Hunter, A, and Dauphinee, J A An approximative colorimetric method for the determination of urea, with an application to the detection and quantitative estimation of arginase Proc Roy Soc, London, s B *97*, 209, 1924

44 Kalckar, H M, and Klenow, H Milk xanthopterin oxidase and pteroylglutamic acid J Biol Chem *172*, 349, 1948

45 Kay, H D Plasma phosphatase in osteitis deformans and in other diseases of bone Brit J Exper Path *10*, 253, 1929

46 Khanolkar, V R, and Chitre, R G Esterase content of serum of mice from certain cancer-resistant and cancer-susceptible strains Cancer Research *2*, 567, 1942.

47 King, E J Preparation of phenolphthalein phosphate J Path & Bact *55*, 311, 1943

48 Kunkel, H G, and Ward, S M Plasma esterase activity in patients with liver disease and the nephrotic syndrome J. Exper. Med *86*, 325, 1947

49 Kutscher, W, and Wolbergs, H · Prostataphosphatase Ztschr f physiol Chem *236*, 237, 1935

50 Levene, P A, and Medigreceanu, F The action of gastro-intestinal juices on nucleic acids J Biol Chem *9*, 375, 1911

51 Markowitz, J, and Hough, H B A study of blood diastases in depancreatized dogs Am J Physiol *76*, 571, 1926

52 Marrack, J Immunochemistry and its relation to enzymes Ergebn d Enzymforsch *7*, 281, 1938

53 Masamune, H: Biochemical studies on carbohydrates IV On an enzyme which catalyses the hydrolysis of biosynthetic osides of glucuronic acid J. Biochem Japan, *19*, 353, 1934

54 McArdle, B The serum choline esterase in jaundice and disease of the liver Quart J Med *9*, 107, 1940

55 McCance, R. A., Widdowson, E M., and Hutchinson, A O: Effect of under-nutrition and alterations in diet on the choline esterase activity of serum Nature *161*, 56, 1948
56 McCall, M L, and Reinhold, J G: Evaluation of clinical significance of serum amylase and lipase determination. Surg, Gynec & Obst. *80*, 435, 1945
57. McCaughan, J M: The values of estimation of the amylase of the blood in the diagnosis of suspected pancreatic disease. Surg, Gynec. & Obst *59*, 598, 1934.
58 McDonald, D, and Odell, L. D · Serum glucuronidase activity during normal and toxemic pregnancy. J. Clin Endocrinol 7, 535, 1947.
59 Mendel, B, Mundell, D B, and Rudney, H. Studies on cholinesterase III. Specific tests for the true cholinesterase and pseudo-cholinesterase· Biochem J *37*, 473, 1943
60 Mendel, B, and Rudney, H: Studies on Cholinesterase. I. Cholinesterase and pseudo-cholinesterase Biochem J. *37*, 59, 1943.
61 Michaelis, L, and Menten, M. L. Die Kinetik der Invertinwirkung Biochem. Ztschr. *49*, 333, 1913
62. Moulton, S H, and Huggins, C.· Esterases of testis and other tissues J. Exper. Med *88*, 169, 1948
63 Northrop, J H · The mechanism of the influence of acids and alkalies on the digestion of proteins by pepsin or trypsin J Gen Physiol *5*, 263, 1922
64. Ohmori, Y · Ueber die Phosphomonoesterase. Enzymologia *4*, 217, 1937.
65 Pauling, L Chemical achievement and hope for the future Am. Scientist *36*, 51, 1948
66 Reid, E., Quigley, J. P, and Myers, V. C. Studies on animal diastases V. Blood and tissue diastases with special reference to the depancreatized dog J Biol Chem. *99*, 615, 1933
67 Roberts, W M · Blood phosphatase and the van den Bergh reaction in the differentiation of the several types of jaundice Brit M J. *1*, 734, 1933
68 Robison, R · The possible significance of hexosephosphoric esters in ossification Biochem J *17*, 286, 1923.
68a Russell, P S, and Huggins, C Colorimetric determination of amylase Ann Surg *128*, 668, 1948
69 Shimkin, M B, Greenstein, J. P, and Andervont, H B · Esterase activity of blood serum of four strains of mice. J Nat Cancer Inst. *5*, 29, 1944
70 Sibley, J A, and Lehninger, A L Unpublished data
71 Somogyi, M Micromethods of the estimation of diastase J Biol Chem *125*, 399, 1938
72 Somogyi, M. Blood diastase in health and diabetes J. Biol Chem. *134*, 315, 1940
73 Spiegelman, S, Lindegren, C C, and Lindegren, G · Maintenance and increase of a genetic character by a substrate-cytoplasmic interaction in the absence of the specific gene Proc Nat Acad Sc *31*, 95, 1945

74 Sumner, J B, and Somers, G F.: Chemistry and Methods of Enzymes, 2nd ed New York, Academic Press, 1947.
75. Susuki, U, Yoshimuri, K, and Takaisha, M Tokyo Imp Univ Coll Agric. Bull 7, 503, 1907.
76. Talalay, P., Fishman, W H, and Huggins, C Chromogenic substrates II. Phenolphthalein glucuronic acid as substrate for the assay of glucuronidase activity J Biol. Chem *166*, 757, 1946
77. Umeno, M.: Studien ueber Phosphatase VI Ueber das Vorkommen der Phosphatase in der Galle und im Pankreassaft Biochem Ztschr *231*, 346, 1931
78. Van Slyke, D. D : The kinetics of hydrolytic enzymes and their bearing on methods for measuring enzyme activity. In · Advances in Enzymology, Vol. 2, p 33 New York, Interscience Publishers, 1942.
79 Warburg, O, and Christian, W Garungsfermente im Blutserum von Tumor-Ratten Biochem Ztschr. *314*, 399, 1943
80 Wohlgemuth, J.: Ueber eine neue Methode zur quantitativen Bestimmung des diastatischen Ferments Biochem Ztschr *9*, 1, 1908
81. Wohlgemuth, J Beitrag zur funktionellen Diagnostik des Pankreas Berl klin. Wchenschr *47*, 92, 1910
82 Woodard, H Q, and Craver, L F Serum phosphatase in the lymphomatoid diseases J Clin Investigation *19*, 1, 1940
83 Zeller, E A, and Birkhauser, H Ueber eine in Vitro-Schwangerschaftsreaktion Schweiz med Wchenschr. *21*, 975, 1940

Plasma Fractionation*

CHARLES A. JANEWAY
Harvard Medical School, and Children's Medical Center, Boston, Massachusetts

Introduction

Blood, which is a suspension of a number of different types of cells in a complex solution, the plasma, has been administered for many different purposes in medical treatment Although whole blood may be an ideal agent to replace combined deficits of both hemoglobin and plasma proteins due to severe trauma, hemorrhage, or malnutrition, its use in many other conditions may be wasteful, ineffective, or even deleterious. For example, in cases of hemophilia with local bleeding, excessive amounts of blood frequently must be given to supply the small amounts of antihemophilic substance actually needed to repair the coagulation defect of the disease If this particular active principle could be purified, concentrated, and stored in stable form, it would be available in time of need in sufficient amounts to reduce the coagulation time without the undesirable overloading of the circulation and inconvenience to both doctor and patient necessitated by multiple blood transfusions This concrete example illustrates the objective of plasma fractionation—to provide the various physiologically active and clinically useful proteins of plasma in as pure, as concentrated, and as stable a form as possible for therapeutic and investigative purposes. It is a logical extension of the development which began when blood was first separated into plasma, to be preserved for future use, and red cells,

* The author's own work in this field has been carried out in close collaboration with the Department of Physical Chemistry, Harvard Medical School, under contracts, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University Currently, these studies are being supported by a grant, recommended by the Panel on Hematology, from the National Institutes of Health of the U S Public Health Service

previously discarded but now frequently used in the replacement therapy of anemia, and is entirely analogous to the trend in the field of pharmacotherapy from the compound empiric prescriptions of earlier days to the use of a single, accurately standardized drug for a particular indication.

Plasma, the fluid matrix in which the cells are suspended and transported throughout the body, differs from the interstitial fluid in its content of proteins, which under normal conditions are largely retained within the vascular system and only pass through the capillary walls in small amounts. These plasma proteins are a mixture of a large number of chemical entities which differ markedly in the concentrations at which they are normally maintained, in the size, shape, and fine structure of their molecules, in the physiologic functions which they perform, and in their sites of production and fate within the body (1–5). Thus, there is a rational basis for their separation, for therapeutic purposes, into fractions, in each of which one or another of the plasma proteins mediating a particular function is specifically concentrated.

DEVELOPMENT OF PLASMA FRACTIONATION

The idea of plasma fractionation is not new. When it was discovered that the injection of hyperimmune animal serums for the treatment of diseases, such as pneumonia and diphtheria, was often followed by serum sickness, efforts were made to separate the therapeutically useful fraction from the other proteins. Many methods have been devised for the concentration of antibodies from hyperimmune animal serums; these include precipitation with neutral salts, alcohol, or specific antigens, and enzymic digestion with heat coagulation of the inert proteins For fractionation of human plasma, such processes are either wasteful, since other proteins are denatured, or impractical, because the method of concentration would lead to the development of pyrogenic material in the solution or could only be carried out on a large scale at prohibitive cost

Before the war, a number of investigators had begun to consider the utilization of animal serum or plasma as a substitute for human plasma, which at that time was difficult to procure in amounts sufficient for possible military needs Wangensteen and colleagues (11, 16) investigated the safety and nutritional value of bovine plasma in man, but encountered a high incidence of immediate reactions.

Keys and co-workers studied the possibility of utilizing the albumin fraction of animal blood (10,14,15) since this fraction is largely responsible for the osmotic activity of blood and since it was found by skin tests that fewer human beings were sensitive to the albumin than to the globulin fraction of heterologous plasma (12,13). Davis and Eaton (9) showed that dogs tolerated an albumin fraction well, although bovine plasma could not be given to dogs without serious immediate reactions.

Thus in 1940, when the National Research Council was requested by the Armed Forces to consider the problem of blood substitutes, it was only natural that studies upon bovine plasma should have been encouraged. The problem was presented to Professor Edwin J. Cohn of the Department of Physical Chemistry of the Harvard Medical School by Drs. Walter B Cannon and David Edsall, and the methods of plasma fractionation were first applied to the separation of bovine plasma into some of its components, in an effort to determine whether one or another of its fractions, particularly albumin, might serve as a plasma substitute in man (6). Rapid progress was possible because of fundamental research that had been carried on for a number of years upon the solubility of proteins and their separation from mixtures (7).

As soon as a relatively pure albumin fraction could be prepared from bovine plasma, comparative studies were initiated upon human plasma It was to be expected that the injection of fractions of animal plasma might be attended with the risk of immediate anaphylactic reactions and delayed serum sickness, but that administration of human protein fractions would avoid this danger Consequently, when the enlistment of volunteer donors by the American Red Cross for the dried plasma program of the Army and Navy showed that blood could be collected on a scale hitherto considered impossible, work on the fractionation of human plasma was pushed vigorously.

The rapid development of the plasma fractionation program was made possible by the cooperation of many agencies—the blood donor service of the American Red Cross, the research funds of Harvard University, the Rockefeller Foundation, and the National Research Council at first, and later of the Federal Government through the Office of Scientific Research and Development, contracts being recommended by the Committee on Medical Research The advice of the Subcommittee on Blood Substitutes of the Na-

tional Research Council was very helpful in guiding and assisting the program. Although most of the large-scale methods used were worked out in the Plasma Fractionation Laboratory and the Department of Physical Chemistry of the Harvard Medical School, with the collaboration of the Division of Biologic Laboratories of the Massachusetts Department of Public Health, chemists, medical scientists, and clinicians from many institutions and many parts of the country were actively associated with this development, which resulted in the fractionation of over two million bleedings and the delivery of large quantities of accepted plasma derivatives from commercial laboratories to the Army and Navy for their use in the field (8). Blood derivatives which are surplus to the needs of the Army and Navy are being returned to the civilian population by the American Red Cross as the agency that originally collected the blood from which they were prepared. The products *of plasma fractionation now seem destined to find an important* place in civilian practice and research as a result of their production by one commercial laboratory and two state public health laboratories, and in consequence of the plan for a national blood program recently adopted by the American Red Cross

Human Plasma Fractions

METHODS OF SEPARATION AND ANALYSIS

The system used in the fractionation of human plasma was designed for practical use on a large scale. Conventional salting-out procedures were impractical for this purpose because of the dialysis necessary to rid the protein precipitate of the high concentration of ions of neutral salts However, by using a volatile organic solvent as precipitant, work could be carried on at subzero temperature, thus minimizing the chance that the more labile protein molecules would be denatured and that pyrogenic substances would form as a result of bacterial multiplication By controlling five different variables, namely: (*1*) ethanol concentration, (*2*) temperature, (*3*) ionic strength, (*4*) hydrogen ion concentration, and (*5*) protein concentration, it has been possible to develop optimal conditions for the precipitation of a particular protein without damage to other proteins remaining in the supernatant. The precipitated protein can be separated by Sharples centrifugation as a wet paste, and from this the small amounts of water and alcohol can be

removed in most instances by the process of drying from the frozen state, which has been so successfully applied to the preservation of plasma, leaving the fraction as a dry powder (Fig. 1) These powders can be stored under optimal conditions until needed Then the powder can be dissolved in a suitable diluent, sterilized by Seitz filtration, and bottled in final containers in the particular state best adapted to the preservation of its physiologic activity. While whole plasma must be kept either frozen or dried to preserve many of its labile components, some of the proteins which appear to be unstable in liquid plasma have proved to be more stable when purified and kept in concentrated solution This may be due in part to separation of a particular protein from enzymes capable of destroying it These methods have a significance considerably beyond the problem they were developed to meet, and represent a distinct advance in the fractionation of biologic systems in general, for example, purification of animal antiserums, hormones, and enzymes (23). Methods utilizing the same general principle (separation with a volatile organic solvent at low temperature) have been developed in other laboratories for the fractionation of human plasma (30) and the purification of viruses (24)

In the development of the fractionation process, satisfactory methods for chemical and physiologic assay of the different components have been extremely important In the case of albumin the problem was simple, since it could be readily identified by its electrophoretic mobility No physiologic test was needed, its osmotic function was known to depend on its chemical properties Similarly, with the immune bodies, available knowledge indicated that they would probably be concentrated in the gamma globulin fraction, but careful tests for a number of antibodies have been made in an effort to follow their concentration in the various globulin fractions Fibrinogen could be followed by its property of forming clots in the presence of thrombin, and the actual percentage of clottable nitrogen is the best index of the purity of a fibrinogen fraction Similarly, prothrombin, thrombin, and fibrinolytic enzyme can be titrated in properly designed *in vitro* systems In other instances, however, the analytical methods for measuring important physiologic properties are so crude that chemical progress has been seriously retarded

Throughout the studies, an effort has been made to characterize the various plasma proteins as accurately as possible by the various

tional Research Council was very helpful in guiding and assisting the program. Although most of the large-scale methods used were worked out in the Plasma Fractionation Laboratory and the Department of Physical Chemistry of the Harvard Medical School, with the collaboration of the Division of Biologic Laboratories of the Massachusetts Department of Public Health, chemists, medical scientists, and clinicians from many institutions and many parts of the country were actively associated with this development, which resulted in the fractionation of over two million bleedings and the delivery of large quantities of accepted plasma derivatives from commercial laboratories to the Army and Navy for their use in the field (8). Blood derivatives which are surplus to the needs of the Army and Navy are being returned to the civilian population by the American Red Cross as the agency that originally collected the blood from which they were prepared The products of plasma fractionation now seem destined to find an important place in civilian practice and research as a result of their production by one commercial laboratory and two state public health laboratories, and in consequence of the plan for a national blood program recently adopted by the American Red Cross

Human Plasma Fractions

METHODS OF SEPARATION AND ANALYSIS

The system used in the fractionation of human plasma was designed for practical use on a large scale. Conventional salting-out procedures were impractical for this purpose because of the dialysis necessary to rid the protein precipitate of the high concentration of ions of neutral salts. However, by using a volatile organic solvent as precipitant, work could be carried on at subzero temperature, thus minimizing the chance that the more labile protein molecules would be denatured and that pyrogenic substances would form as a result of bacterial multiplication. By controlling five different variables, namely (*1*) ethanol concentration, (*2*) temperature, (*3*) ionic strength, (*4*) hydrogen ion concentration, and (*5*) protein concentration, it has been possible to develop optimal conditions for the precipitation of a particular protein without damage to other proteins remaining in the supernatant. The precipitated protein can be separated by Sharples centrifugation as a wet paste, and from this the small amounts of water and alcohol can be

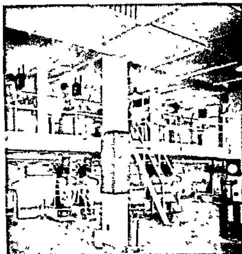


Fig 1C. Interior of cold room in large-scale commercial processing laboratory (8,36i) Everything is operated in closed system, whole process can be controlled by very few people.



Fig 1D Battery of driers in commercial processing laboratory (8).

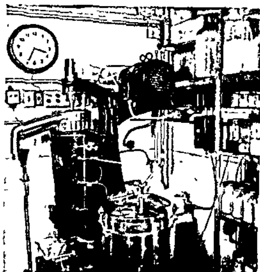


Fig 1A Harvard Pilot Plant Detail showing cold room, Sharples centrifuge, and stock of dried powders in bulk storage



Fig 1B Harvard Pilot Plant Detail showing method of adding buffer solution to protein in alcohol-water mixture in glass-lined tank

plasma The plasma is then drawn off and pooled. The value of the remaining packed cells for replacement therapy in anemia has not been fully appreciated and should be emphasized, since their administration, instead of whole blood, to patients with a deficit of hemoglobin but not of plasma proteins is effective, rational, and necessary in order to reduce losses of blood stored beyond the expiration date and to effect adequate economies in the use of blood in a civilian blood program. The plasma from 200 to 5,000 bleedings may be used to make up the initial pool, depending on the size of

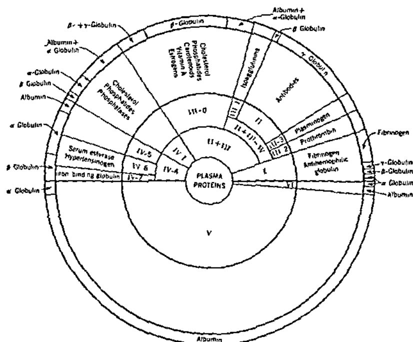


Fig 2 Separation of plasma proteins into fractions (36a)

the run. Ordinarily, the plasma is pooled at random; but if isoagglutinins are to be derived from the process, the blood group of each donor must be determined and bloods of one group only pooled. The huge size of the initial plasma pools is an important factor in assuring their comparative uniformity, although at first it was felt that the chance of contamination by the hepatitis agent or by other viruses would be much greater than with the usual plasma pools of

methods suitable for the study of the proteins. These are briefly listed below, but the reader who wishes to get a clearer understanding of their significance should consult the paper of Cohn and associates (22).

Electrophoresis. The Tiselius apparatus has an ingenious optical system, by which the mobilities of proteins in an electric field may be visualized and recorded graphically This makes it possible to determine the relative proportions of different components in a mixture like plasma fairly rapidly. Consequently, it has been an extremely convenient tool for following the progress of fractionation, particularly since in the case of albumin and gamma globulins, the desired physiologic function was known to be associated with a distinct electrophoretic component

Ultracentrifugation. The method of sedimentation by centrifugal force at extremely high speeds, originally developed by Svedberg for the measurement of molecular weight, has not only proved useful for this purpose, but also in the study of subtle changes in proteins, where molecular cleavage or aggregation may have taken place without other detectable alterations

Measurements of Osmotic Pressure, Diffusion, Viscosity, Double Refraction of Flow, and Electric Moment These various measurements have been used to gain insight into details of molecular structure, such as size and configuration, which help to elucidate the behavior of particular proteins in the body (33).

Methods of Organic Chemistry. These methods have been used to study the composition of proteins in terms of their fundamental chemical units *Analyses for carbohydrate and lipids,* such as cholesterol, indicate the amounts of non-nitrogenous constituents embodied in the molecules of certain types of protein *Amino acid analyses* (17) of those proteins which have been isolated in relative purity provide insight into the chemical differences on which variations in physiologic function, nutritional value, and antigenic behavior depend *Immunologic* methods have revealed differences not otherwise detectable between molecules with great chemical similarity, such as human and bovine serum albumin (29)

PLASMA FRACTIONS

The process of fractionation begins with separation of the red cells from the plasma by centrifugation, as in the production of

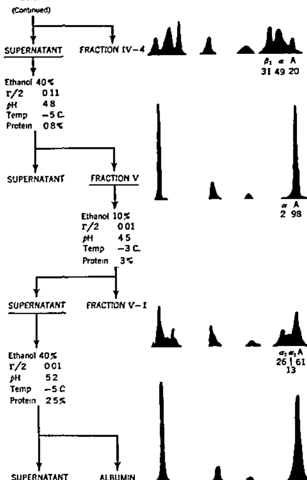


Figure 3B

The present system of fractionation, revised in the interest of preserving the integrity of certain stable components and of obtaining greater yields, results in the separation of plasma into six major fractions (Method 6) (23). The conditions for their separation, their proportions, and components are shown in Figures 2 and 3

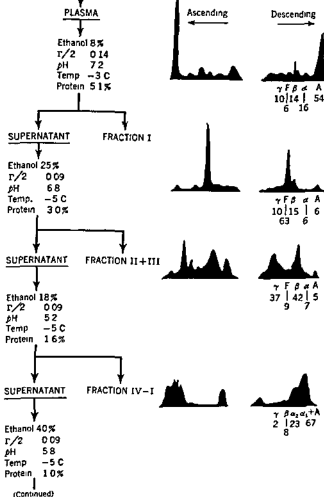


Fig 3A Method 6 Present method used in fractionation of normal human plasma, showing electrophoretic composition of the fractions (23) Figure 3B is a continuation of Figure 3A

rom 10 to 50 bloods So far, there has been no evidence to subtantiate this fear in the case of two products, serum albumin and ormal serum gamma globulin

small for detection. Of the protein components of *complement* C'1 is found in Fraction II + III and C'2 in IV-1 (34) Certain enzymes such as *serum esterase* (18) and *alkaline phosphatase* (37) have been identified in this fraction The lipoproteins, which are both α- and β-globulins, are little understood but consist of proteins to which various lipids, including phosphatides, carotenoids, steroid hormones, and cholesterol, are bound. Because they are labile and difficult to handle, much of the work in the fractionation of plasma so far has been aimed at ridding the nonlipid fractions—albumin, immune globulin, and fibrinogen—of their presence In the future they should command increasing attention, because of our comparative ignorance concerning their properties and functions.

Fraction V. This fraction contains 47 per cent of the total plasma proteins and 80 per cent of the total *albumin* in a state of 97 per cent purity. When dry, it is a stable white powder. Methods for the recovery of the albumin lost in other fractions are being developed.

Fraction VI. Only about 1 per cent of the total plasma protein is contained in this fraction. It is merely the supernatant, containing a little protein and solutes of low molecular weight which remain after 99 per cent of the plasma proteins have been precipitated.

Subfractions of II + III

The original purpose of the plasma fractionation program was to prepare human serum albumin, first for clinical trial, and, after its acceptance, for use by the Armed Forces. In so doing, the globulins were stored as Fraction II + III until their further purification could be undertaken The wisdom of salvaging this valuable material has been amply demonstrated with the progress of studies on the subfractionation of II + III The first methods were not inclusive; prothrombin was lost in the preparation of isoagglutinins, and vice versa Subsequently, new methods developed by Oncley *et al* (32) and Deutsch *et al* (26) made it possible to prepare all the known useful components, with excellent yields, by a single process. This method results in a number of subfractions which will be described below, and which are represented in Figure 4.

Fraction III-0 is first taken off by washing with cold alcohol. This removes the lipoproteins of Fraction II + III which are con-

The order in which the fractions are numbered and taken off correlates with their solubility in ethanol–water mixtures, but as the concentration of ethanol is raised, the *p*H, temperature, ionic strength, and protein concentration are also adjusted to provide optimal conditions for the separation of each fraction without denaturation of the proteins remaining in the supernatant

Major Fractions

Fraction I This fraction contains approximately two-thirds of the plasma fibrinogen and only 5 per cent of the plasma proteins. It consists of 60 to 70 per cent fibrinogen and a mixture of other components, including the *antihemophilic globulin* and another protein the solubility of which is markedly dependent on temperature. Although preparations with 98 per cent clottable nitrogen can be prepared, and some antihemophilic activity persists after the fibrinogen of fraction I is removed by coagulation with thrombin, a satisfactory method for separation of fibrinogen from antihemophilic globulin has not yet been developed

Fraction II + III The two combined are a large fraction containing many important globulins. *lipoproteins, isohemagglutinins, prothrombin, and antibodies* It comprises approximately 28 per cent of the total plasma proteins and is chiefly made up of β- and γ-globulins It is not used as such, but is divided into a number of subfractions in order to separate its several important components

Fraction IV Comprising 15 per cent of the total proteins, it was originally taken off as a single fraction containing the α- and β-globulins not precipitated in Fractions I and II + III. It is now taken off in two parts *Fraction IV-1* contains the lipoproteins of this fraction, principally α-globulins, while *Fraction IV-4* contains mainly those α- and *β-globulins* that are soluble in water, have relatively low molecular weights, and most nearly resemble albumins in their properties The proteins of Fraction IV and the lipoproteins in Fraction II + III present a challenge to investigation in the future *Hypertensinogen*, the substrate on which renin, produced by the kidney under conditions of ischemia, acts to release hypertensin (angiotonin), the vasoconstrictor substance responsible for renal hypertension, is found in Fraction IV-4 (25), as is the *thyrotropic hormone* (27), which is present in plasma in amounts too

II + IIIW, and their further subfractionation yields four fractions, as follows.

Fraction II, containing 70 to 80 per cent of the γ-globulins of the plasma in 98 per cent purity, is the fraction in which the *immune bodies* are concentrated. It is a stable white powder when dried and makes an almost water-clear solution Two γ-globulin fractions (II-1,2 and II-3) of different solubilities can be prepared. Although both contain antibodies in high concentration, the distribution of individual antibodies between them is somewhat different Proof that sufficient measles protective antibody is recovered in Fraction II-3 has now been obtained, so that these two γ-globulin fractions may now be separated together, increasing the yield from 55 per cent (II-1,2) to approximately 70 to 80 per cent.

Fraction III-1, chiefly β_1-globulin, contains the isohemagglutinins. It, too, is stable as a dry white powder, which readily dissolves for use *The Rh typing globulin* is separated in this fraction when plasma containing Rh agglutinins is used as starting material

Fraction III-2, largely β_2-globulins, is the fraction containing *prothrombin*. Before being processed finally, the unstable prothrombin is usually converted to thrombin by the addition of a small amount of thromboplastin (from human placenta) and calcium ions It is stable when dried from the frozen state, giving a white powder, readily dissolved in physiologic saline to form a slightly opalescent solution Chemical differences between prothrombin and thrombin, other than the instability of the former, have not been elucidated * Actually, prothrombin makes up a very small portion of the total protein in III-2

Fraction III-3 is a rather heterogeneous fraction containing the fibrinogen of Fraction II + III. When this fraction is coagulated by the addition of a small amount of thrombin, the resultant clot is lyzed in a short period at room temperature, due to the formation of plasmin (*fibrinolytic enzyme*) (19) If such material is allowed to stand in the cold room for a considerable period, this fibrinolytic activity will increase as more and more enzyme is activated from the inactive form (plasminogen) in which it is normally present in plasma (35)

Thus, from Fraction II + III it is possible to prepare the immune globulins, prothrombin and thrombin, fibrinolysin, and a β_1-lipoprotein fraction with extraordinary properties If properly

* A recent report suggests a considerable difference in molecular weight (195)

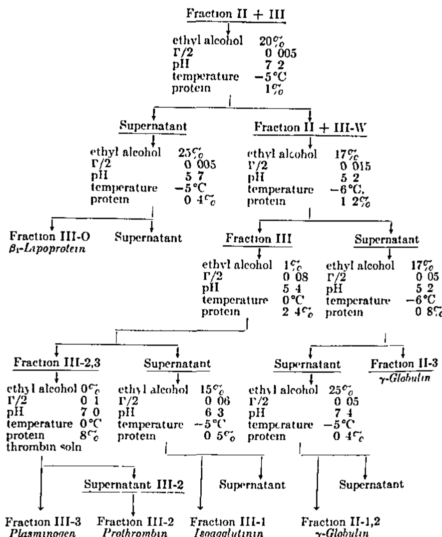


Fig 4 Method 9 Present method for subfractionation of Fraction II + III (32,36a) Separation of prothrombin, isoagglutinins, plasminogen, and γ-globulins

centrated in this fraction as β-globulins, both euglobulins and pseudoglobulins The molecules of a β_1-lipoprotein of high molecular weight in this interesting fraction contain as much as 75 per cent lipid, with a correspondingly high nitrogen factor and low density The proteins remaining behind after the washing step are known as

000). Among them, several physiologically active components have been recognized: hypertensinogen (25), serum esterase (18), and a metal-binding protein (36). During the past year, it has been possible to concentrate the latter protein in Fraction IV-7 and crystallize it, thus proving that it is a β_1-globulin (21). Its capacity to bind metals will account for all the iron present in plasma, and again indicates how specific the reactions may be between proteins



Fig 5B Crystals of human serum albumin crystallized with mercuric chloride (8,20,192) Ethanol, 10 per cent, $HgCl_2$, 0 01 per cent; Γ/2, 0 05, *p*H, 5.5

and small molecules Recent studies on the serum albumin fraction (Fraction V) have yielded equally interesting results Cohn, Hughes, and Weare (20) have succeeded in crystallizing serum albumins from ethanol–water solutions of Fraction V, with the addition of small amounts of decanol Hughes (28) has also demonstrated that a crystalline mercuric salt, in which two albumin molecules are linked through one atom of mercury, can be obtained when a small amount of mercuric chloride is added to a solution of Fraction V or of crystalline human serum albumin prepared by the

selected plasma is used, the isohemagglutinins or Rh typing globulin may also be obtained

Subfractions of IV-1, IV-4, and V

Work which is now being actively pursued in Professor Cohn's laboratory is aimed at the separation and characterization of the many interesting components in Fractions IV-1 and IV-4. Since

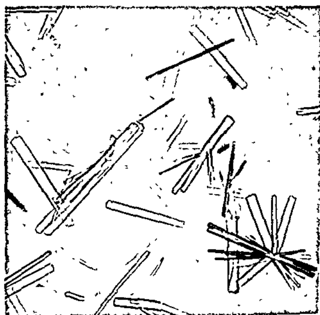


Fig 5A Crystals of human serum albumin crystallized with decanol (8,20,192) Ethanol, 25 per cent, decanol, 0 1 per cent, Γ/2, 0 1, pH 5 5

this work is still in progress, little of a definite nature can be stated at present But a clearer picture of the specific nature of plasma protein interactions and of their binding of smaller molecules is emerging. The proteins of Fraction IV are chiefly α- and β_1-globulins Those in IV-1 are lipoproteins, bearing certain of the steroid hormones and such substances as Vitamin A and the carotenoids in their lipoid portions Those of IV-4 are water-soluble α- and β-globulins of relatively low molecular weight (average 90,-

acid analyses of fibrinogen and fibrin fail to demonstrate significant differences between them (17). In plasma, fibrinogen is normally present in a concentration of 0.25 per cent. Because of its large molecular size, fibrinogen contributes little to the osmotic activity, but by virtue of its asymmetry makes the greatest contribution to the viscosity of plasma (33). As recovered in Fraction I, fibrinogen makes up approximately two-thirds of the protein present; it may be satisfactorily used in this state of partial purification, although it has been possible to obtain far greater purity in the laboratory.

Prothrombin, which is recovered in Fraction III-2, is extremely unstable, and thus has not been prepared for therapeutic use. It may be converted rapidly to *thrombin* in the presence of calcium ions by the addition of a small amount of thromboplastic material extracted from human placental tissue. This thrombin is sufficiently stable to permit satisfactory sterilization by filtration, but must be frozen and dried to preserve its activity when stored. When reconstituted with physiological saline solution, it dissolves readily and has marked thrombic activity. Whereas fibrinogen is needed in fairly large amounts for the clinical uses to be described but is present in only small amounts in plasma, adequate thrombin for all purposes is readily obtained by fractionation.

The *interaction of thrombin with fibrinogen* produces a coagulum of fibrin, but the conditions under which this interaction is allowed to take place determine the properties of the resulting clot (45,46). It is thus possible to develop products specifically devised to meet definite clinical needs. Although the clinical applications of discoveries in this field are chiefly in surgery, their consideration here is justified by their general interest. The factors which affect the characteristics of the clot have been carefully studied. Obviously the concentration of fibrinogen is very important, but the *p*H of the solution in which the clot is formed has a profound effect on the properties of clots formed at any given protein concentration. Clots formed at more alkaline *p*H (8.0) tend to be clear, more friable, and synergize but little. In contrast to these Type A clots, Type B clots formed at more acid *p*H (6.3) are somewhat opaque, have greater tensile strength and synergize markedly (46). In a recent study (51) it has been possible to show, by means of the electron microscope, that the fibrils of a Type B clot are of greater diameter than those of a Type A clot, indicating side-to-side as well as end-

decanol method (Fig 5). Fraction V-1, a small subfraction made up of the 2 per cent of globulin in Fraction V, has been found to account for certain properties formerly attributed to albumin. Thus, the protein-bound bilirubin of plasma, which gives the indirect van den Bergh reaction, is recovered in this fraction, where it appears to be bound to an α_2-globulin (31).

Clinical Use of Plasma Fractionation Products

FIBRINOGEN, THROMBIN, AND THEIR PRODUCTS

The *fibrinogen* molecule is large and exceedingly asymmetrical, with a length roughly 27 times as great as its equatorial diameter and a molecular weight of approximately 400,000 (Fig. 6). Because

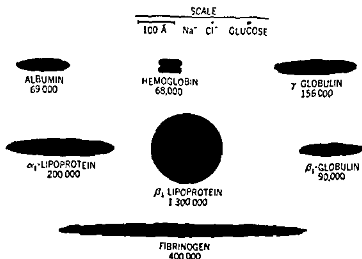
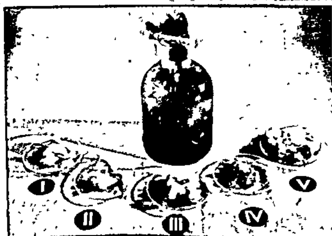


Fig 6 Relative dimensions of various proteins (as revised by Oncley) (8,192)

of the marked asymmetry, solutions of fibrinogen are extremely viscous and exhibit characteristic double refraction of flow (44) These properties are exactly those which fit it to serve as the matrix of the blood clot, in which, as a result of the conversion of fibrinogen to fibrin by the action of thrombin, its long molecules are linked together into elongated fibrils (46). Although the physical basis of coagulation has been established, details of the chemical reactions which bring this about are as yet not understood Amino





Fibrinogen and Thrombin
Fractions I and III-2



Fibrin Foam and Thrombin
Fractions I and III-2



Fibrin Film
Fractions I and III-2



Serum γ-Globulin
Fraction II



Isoagglutinins
Fraction III-1



Serum Albumin
Fraction V

By *Life* photographer Fritz Goro Copyright *Time, Inc*

Fig 7 Products derived from fractionation of human blood plasma (8) Above Bleeding bottle containing one donation and the amount of wet paste in each of the major fractions as obtained by an earlier method of fractionation Below Some of the final products, as packaged for distribution during the Red Cross Army–Navy program

to-end aggregation of molecules in the opaque, tough clots. Fibrinogen may be clotted *in vitro* by the action of added thrombin under controlled conditions, such that products of very different properties are obtained. Thus, a coagulum formed with a spongy consistency due to entrapped air is known as a *foam* (41); if it is formed *under mechanical pressure and in the presence of a plasticizing* agent, a thin sheet or *film* (Fig 7) is produced (47). By varying the proportions of the reacting proteins and plasticizers, films of different thickness, tensile strength, and elasticity may be obtained, which are further altered by the methods used for sterilization (48). In addition to foams and *films*, plastics may be produced from fibrinogen and plasticizer without thrombin, coagulation being induced by heating the protein-plasticizer mixture in a mold of the desired shape (45) Thus, by means of chemical manipulation products can be developed with an exceedingly wide variety of physical properties from those proteins which produce coagulation in a relatively uniform manner in the body The possible uses of such products in surgery would appear to be greater than those already established for them.

Since the basic constituents of these products are human proteins they should be expected to produce minimal tissue reactions in the human body Nevertheless, the heating manipulations involved in producing sterile final products might well denature these proteins sufficiently to render them foreign and irritating to the tissues (59) Consequently, their application to human surgery has been preceded by extremely careful experiments and pathological studies of the tissue reactions they induce in animals *(38,39,53)*. The rates at which the various products of fibrinogen are absorbed in the body will depend on the extent to which the protein has been altered in the process of production so as to render it resistant to the lytic enzymes which may attack it The presence of small amounts of plasminogen in preparations of Fraction I leads to gradual lysis of clots, unless certain substances, such as glycerol, are present or heat is applied, as it is in the sterilization of foams and *films*.

Surgical Uses of Fibrinogen Products

Fribrinogen has been used locally in several ways as an adjunct to surgical procedures in which a physiologic coagulum of homologous material is desired

of human plasma by clotting Fraction I with thrombin under such conditions, that when the product was dried its structure consisted of dense strands of fibrin with air spaces of many sizes between them This *fibrin foam*, after sterilization, is a dull, dry, brittle mass resembling a piece of stale bread. It is packaged with a small vial of dried human thrombin and a 30 cc vial of physiologic saline solution For use, the thrombin is dissolved in the saline solution and the pieces of foam are soaked in this solution The foam rapidly becomes wet, loses its brittleness, and becomes rubbery and compressible When pieces of foam soaked in thrombin are applied with slight pressure to an oozing surface, blood wells up into the interstices of the foam; there it is coagulated by the thrombin, and the foam becomes adherent Since the foam consists of human protein, it may be left in place to be absorbed slowly

Ingraham, Bailey, and Nulsen (56) have demonstrated conclusively that fibrin foam, when used as a local hemostatic agent, produces a minimal degree of tissue reaction, especially when compared with muscle in stanching bleeding over the surface of the brain. Fibrin foam has been used in a large number of neurosurgical operations and has proved an invaluable method for the control of hemorrhage within the cranial cavity (54,67) In military surgery, it has been particularly useful in stopping bleeding from traumatic rupture of the major dural sinuses As a hemostatic agent, it has one important application of great interest to medical men—the control of external hemorrhage, as after dental extraction, in hemophilia patients (58). In addition to the latter application, and its use in neurosurgery, fibrin foam with thrombin has been used as a hemostatic in situations in general surgery in which local bleeding presents a problem (40,63). Foams of various types may well be devised to meet the particular specifications laid down for a specific purpose

A similar absorbable sponge of gelatin, for use with bovine thrombin (57), has recently been developed and tested as a hemostatic agent (61) Based on comparisons of this product with fibrin foam that have been made in a number of surgical clinics, there is general agreement that the gelatin sponge is about as satisfactory.

Fibrin Film The wide variety of fibrin films which can be produced by the technics of Ferry and Morrison suggests that these products might be utilized in many surgical conditions. However,

Removal of Renal Calculi Dees (43) has pointed out that the operative removal of multiple calculi from the renal pelvis is frequently incomplete and difficult, since small calculi often escape detection. To overcome this difficulty he devised an operation, in which, after lavage of the renal pelvis with a wetting agent, fibrinogen solution is injected and coagulated by a small amount of thrombin. The coagulum, which entraps all the calculi, may then be lifted out in one piece, thus avoiding the danger of crumbling calculi and effecting, in most cases, a complete removal of all stones present.

Fibrinogen–Thrombin Mixtures as Dressing for Superficial Wounds If a mixture of powdered fibrinogen (Fraction I) and thrombin is applied to a denuded, oozing surface, a coagulum forms as the serous fluid wets the dry protein mixture. Bacteriostatic drugs may be incorporated in such powders, if desired. Such mixtures have been used experimentally by Hawn and associates (50) and have been demonstrated not to interfere with the normal processes of repair Another clinical application of these proteins is to form a "physiologic glue" to make skin grafts adhere to the surface to which they are applied, a method which has had limited trial (42).

Fibrin Foam and Thrombin. The need for a satisfactory hemostatic agent to control hemorrhage in sites in which the ordinary mechanical methods of hemostasis are unsuitable has long been felt in all branches of surgery, but particularly in neurosurgery. In recent years, preparations of thrombin have been made available. bovine thrombin purified by the method of Seegers (64) and rabbit thrombin ("clotting globulin") prepared by the method of Parfentjev (60). Unfortunately, thrombin alone, although it induces rapid coagulation of whatever fibrinogen is present, requires that pressure be applied through the medium of a sponge or tampon to effect satisfactory hemostasis. If ordinary cotton or gauze is used as tampons, they must be removed, often pulling away the clot with them. Putnam (62) and Frantz (49) deserve credit for the introduction of an absorbable type of sponge made of oxidized cellulose which, when left *in situ,* is gradually absorbed with a minimum of tissue reaction. This type of sponge was first soaked in a solution of thrombin, but has been found to have hemostatic properties when used alone.

Bering (41) developed an absorbable tampon from the protein-

FRACTION I (ANTIHEMOPHILIC GLOBULIN) IN HEMOPHILIA

Studies in hemophilia, carried on chiefly at the Thorndike Memorial Laboratory by Minot, Taylor, and their associates in the last few years, have demonstrated that the disease is due to a deficiency of a "globulin substance" present in normal human plasma. When this "globulin substance" is added to hemophilic plasma, the clotting time is reduced to normal, and when it is injected into a patient with hemophilia the same phenomenon occurs *in vivo*. Presumably the efficacy of fresh whole blood or plasma transfusions in reducing the prolonged coagulation time characteristic of patients with this disease is due to the presence of this globulin in the material transfused (69,70,73,74).

Studies of the fractions of normal human plasma by Taylor and associates (75) have demonstrated the presence of a substance in several fractions, but principally in Fraction I, which will accelerate the clotting of hemophilic plasma *in vitro*. Attempts to concentrate the antihemophilic globulin from this fraction by separating it from fibrinogen have been unsuccessful thus far, although the nonidentity of fibrinogen with the antihemophilic globulin seems assured by the fact that after coagulation of the fibrinogen in Fraction I, the supernatant retains some antihemophilic activity. Therefore, preliminary studies were begun on the effectiveness of Fraction I in controlling episodes of bleeding in patients with hemophilia. The sterile dry powder, when reconstituted for use with saline and injected intravenously, will promptly reduce the coagulation time in patients, just as it does *in vitro*. In a manner quite analogous to the response to whole blood or plasma, the clotting time soon begins to increase again, but an effect is noticeable for 12 to 24 hours after doses of 200 mg of protein in adults. The material has already been used by Minot and co-workers (71,72) and by Diamond (68) in a considerable number of patients with very satisfactory results in most cases An injection of 5 cc of potent Fraction I (200 mg) in children is usually as effective as the transfusion of approximately 50 cc of fresh plasma, both in reducing clotting time and controlling hemorrhage (Fig. 8) The dose frequently must be repeated several times in the first few days of a period of crisis, but the convenience of administering a substance in small volume which may be kept on hand, ready for use when needed, is hard to over-

clinical and experimental trials to date have been largely concerned with their use in repairing defects of the dura mater, a particularly pressing problem because of the numerous head injuries incurred in battle Any dural substitute must have sufficient strength to serve as an adequate replacement for the tough dural membrane, it should be easy to manipulate at operation, and above all, it should prevent formation of adhesions between the brain and its overlying tissues. Study of the latter problem requires that experimental and clinical observations cover a period of many months, in order to make sure that what appear to be favorable results are not followed by the development of convulsions due to cortical irritation.

Such long term experiments have been carried out in monkeys by Ingraham, Bailey, and Cobb (55), the animals being sacrificed at periods up to 6 months from the time the dural defect was produced and the film implanted These studies have been carried out with fibrin films as originally prepared, and subsequently with films sterilized by steam for large-scale production Their results indicate that films persist in the body for periods up to 6 months, but during this time connective tissue grows around the film, gradually replacing it, without formation of adhesions to the underlying brain These striking experimental results have been duplicated in the clinic Films have been used to repair dural defects in a number of patients In many of these, re-exploration at a subsequent date made it possible to examine the state of the films Their fate in the human body and their ability to prevent the formation of meningocerebral adhesions in man confirm the results obtained in monkeys As now produced, film comes sterilized in a glass tube, which may be broken like an ampule, allowing the film to be dropped into a vessel of sterile saline Within a few minutes, the dry, brittle film absorbs water, becomes less opaque, and regains its elasticity It may then be readily cut to any desired size and shape and slipped into place with forceps. Films may be sutured, but sutures do not appear to be necessary in most instances With the development of similar types of satisfactory films (52) from synthetic plastics, it is possible that fibrin film, like foam, may be less critically needed Recently, Swenson and Gross (66) have utilized fibrin films as tubes to aid in the anastomosis of blood vessels The use of films and clots in nerve suture, although hopeful (65) in animals, gave less satisfactory clinical results

of methanol. The method was then adapted to ethanol fractionation (83), and several preparations of anti-A (from B plasma) and anti-B (from A plasma) proved fairly satisfactory (77). Continued modification of the method finally made it possible (32) to recover prothrombin, isohemagglutinins, and gamma globulin from the same lot of Fraction II + III. However, anti-A preparations were not always sufficiently potent to detect the weakly aggultinable A_2 and A_2B cells Melin (80) was able to develop a method for the production of anti-A isoagglutinins by fractionation of O plasma and removal of anti-B by absorption with B cells. This method had two advantages; first, the resultant anti-A isoagglutinin reacted well with cells containing A_2 agglutinogen, and second, equal quantities of anti-A and anti-B could be prepared by pooling the plasma of all O bloods and all A bloods, respectively, since both O and A donors are encountered in approximately equal proportions. Isoagglutinin preparations of this type will give prompt and readily observed slide agglutination of incompatible cells in a minute or less

Similar methods have been applied to preparation of an anti-Rh globulin from the pooled serum of Rh negative individuals who had been sensitized by pregnancy or transfusions of Rh positive blood (81). Great difficulty was at first encountered because of the presence of blocking antibodies; then it was shown that when the agglutination was carried out with a concentrated cell suspension in a protein-rich diluent instead of in saline the blocking effect disappeared (76,78). Fraction V of bovine plasma has turned out to be a very satisfactory diluent for use in Rh typing.

IMMUNE BODIES

Preparation of Gamma Globulins

When plasma fractionation was first begun for the production of serum albumin, the globulin fractions were stored for possible future use. A survey under the direction of Drs A. R. Dochez and W. C. Boyd indicated that those antibodies for which satisfactory immunologic tests existed and which might be expected to be present in normal adult plasma were quantitatively precipitated in Fraction II + III in a concentration 8 to 10 times that in the average plasma pool (94). This crude fraction was successfully used by Stokes, Maris, and Gellis (119) for the prophylaxis of measles.

estimate. Intramuscular injection may also be used, but it is accompanied by considerable pain and is less reliable, because of variations in absorption. Further studies should make it possible to separate this substance from the other proteins of Fraction I, thus

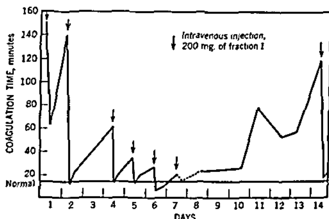


FIG 8 Use of Fraction I to reduce coagulation time to normal in a patient with hemophilia (courtesy Drs L K Diamond and W. Borges).

further simplifying its administration and avoiding a needless waste of valuable fibrinogen

ISOHEMAGGLUTININS AND Rh TYPING GLOBULIN

As the importance of whole blood transfusion in resuscitation of the wounded came to be recognized by those responsible for the medical services of the Armed Forces, the need for dependable readily obtainable blood grouping reagents was emphasized Moreover, with the increasing use of transfusion in civilian medicine, particularly of conditioned group O blood (79), and with the appreciation that Rh sensitization occurs frequently enough to demand routine Rh typing of donor and recipient, this need becomes even more apparent.

Concentration of isohemagglutinins from the pooled plasma of patients of a single blood group was achieved by Thalhimer and Myron (84), using ammonium sulfate precipitation, and subsequently by Pillemer (82) at the Army Medical School by the use

of methanol. The method was then adapted to ethanol fractionation (83), and several preparations of anti-A (from B plasma) and anti-B (from A plasma) proved fairly satisfactory (77). Continued modification of the method finally made it possible (32) to recover prothrombin, isohemagglutinins, and gamma globulin from the same lot of Fraction II + III. However, anti-A preparations were not always sufficiently potent to detect the weakly agglutinable A_2 and A_2B cells. Melin (80) was able to develop a method for the production of anti-A isoagglutinins by fractionation of O plasma and removal of anti-B by absorption with B cells. This method had two advantages; first, the resultant anti-A isoagglutinin reacted well with cells containing A_2 agglutinogen, and second, equal quantities of anti-A and anti-B could be prepared by pooling the plasma of all O bloods and all A bloods, respectively, since both O and A donors are encountered in approximately equal proportions. Isoagglutinin preparations of this type will give prompt and readily observed slide agglutination of incompatible cells in a minute or less

Similar methods have been applied to preparation of an anti-Rh globulin from the pooled serum of Rh negative individuals who had been sensitized by pregnancy or transfusions of Rh positive blood (81). Great difficulty was at first encountered because of the presence of blocking antibodies; then it was shown that when the agglutination was carried out with a concentrated cell suspension in a protein-rich diluent instead of in saline the blocking effect disappeared (76,78). Fraction V of bovine plasma has turned out to be a very satisfactory diluent for use in Rh typing.

IMMUNE BODIES

Preparation of Gamma Globulins

When plasma fractionation was first begun for the production of serum albumin, the globulin fractions were stored for possible future use. A survey under the direction of Drs. A. R. Dochez and W. C. Boyd indicated that those antibodies for which satisfactory immunologic tests existed and which might be expected to be present in normal adult plasma were quantitatively precipitated in Fraction II + III in a concentration 8 to 10 times that in the average plasma pool (94). This crude fraction was successfully used by Stokes, Maris, and Gellis (119) for the prophylaxis of measles.

However, Fraction II + III contains many components—alpha, beta and gamma globulins; antibodies; isoagglutinins; prothrombin; and other physiologically active substances. Hence, further subfractionation must be carried out to purify the gamma globulins and separate them from the less stable alpha and beta globulins. Fraction II, in which the former have been concentrated, originally contained about 85 per cent gamma globulin, the remainder being beta globulin, which resulted in a solution of considerable opalescence and relatively high cholesterol content. As studies continued, the preparation was improved until Fraction II now contains 95 to 99 per cent gamma globulin, yielding an almost water-clear solution with an extremely low cholesterol content (127).

The gamma globulin molecule, with a molecular weight of 156,000, is both larger and more asymmetric than the albumin molecule, its diameter is slightly smaller, and its length is over twice as great. *Consequently, its solutions are considerably more viscous, and exert* very much less osmotic pressure, because of the lower net charge and larger size of the molecule. This is fortunate, since this fraction undergoes the greatest relative increase during certain diseases and after hyperimmunization. Gamma globulin is distributed in solution, because it is quite stable. Studies have shown that glycine and sugars enhance its stability, and hence isotonic glycine (0.3 molar) is now the standard diluent (32)

Antibody Activity of Gamma Globulins

In standard preparations of gamma globulin solution, containing 16.5 per cent protein, of which 95 per cent or more is gamma globulin, there is 25 times as much gamma globulin per unit volume as in the usual plasma pool. With two exceptions, all the antibodies found in Fraction II + III actually appear to be concentrated from 15 to 30 times in Fraction II (94) (Fig 9). The two exceptions are the isohemagglutinins (and anti-Rh globulin) and typhoid O agglutinin, both of which were concentrated in Fraction III-1 by the original methods of subfractionation, and which are now concentrated in III-1 and II-3 respectively (Fig 4) There is considerable theoretic interest in the fact that agglutinins for two different antigens of one organism should be separable on a basis of solubility. The "H" agglutinin for the flagellar antigen of the typhoid

bacillus is found with most of the other antibodies in Fraction II-1,2, whereas the O agglutinin for the somatic antigen is concentrated in Fraction II-3. To what extent antibodies to other antigens may be separated from one another by methods of fractionation rather than by immunologic methods remains to be determined.

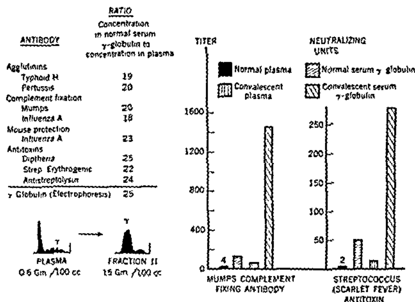


Fig 9 Concentration of antibody in Fraction II (gamma globulin) derived from normal plasma and from convalescent plasma in mumps and scarlet fever Based on work of Dr J F Enders and Miss Julia Sullivan (193)

Unfortunately, it is not possible to measure the potency of gamma globulin preparations *in vitro* against the two diseases for which they have been principally used in man—measles and infectious hepatitis In neither case does a satisfactory laboratory test of immunity exist at the moment It is necessary to rely on the probable immunity of the bulk of adults in this country, controlling the potency of each preparation by insisting on an adequate concentration of gamma globulin, as determined by electrophoretic analysis and measurement of the total protein concentration, and on satisfactory levels of diphtheria antitoxin, typhoid H agglu-

tinin, and influenza A neutralizing antibodies as compared to a standard preparation. In some preparations, field tests against measles have been carried out as a final assay of potency and have indicated a high degree of uniformity in the concentration of measles antibody.

Normal human serum gamma globulin (immune serum globulin, human) has been proved to contain those antibodies present in the pooled plasma of normal adults of our urban population in a concentration approximately 25 times that of the plasma Each cubic centimeter of gamma globulin solution is the equivalent in antibody activity of 25 cc. of pooled adult plasma. For use in the prophylaxis and treatment of disease, such a solution has certain distinct advantages· (*1*) As a normal component of human plasma, it should not give rise to serum sickness. (*2*) It is theoretically possible to give 25 times as much antibody as could be given in pooled plasma; (*3*) It is possible to administer the antibody activity of human plasma intramuscularly in small volume; (*4*) By virtue of its stability, it becomes possible to keep this antibody activity available in convenient vials ready for use over long periods; (*5*) Being derived from human blood, it contains certain antibodies which it has not yet been possible to produce in animals The most important antibodies which have so far been demonstrated in normal human serum gamma globulin are shown in Figure 9.

Clinical Use of Normal Human Serum Gamma Globulin

Safety. In clinical practice, gamma globulin is administered by intramuscular injection, it has not been possible to prepare material which is satisfactory for intravenous use (89). The incidence of all reactions in 2,738 intramuscular injections has been 1 2 per cent, most of which consist of local tenderness at the site of injection. Large doses of more than 10 cc are frequently followed by soreness of the muscle for a number of hours, on rare occasions, fever, malaise, and headache are noted. One patient developed angioneurotic edema of the face the day after injection (105). Two instances of *possible* serious reactions have come to our attention in Massachusetts, where many thousand injections have been given. One is a case of neuromyelitis optica occurring in a young woman 24 hours after an injection, the other, a case of the nephrotic syn-

drome in which edema was first noted a few days after an injection.* It has been the general experience of physicians who have used placental extract (immune globulin) for measles prophylaxis that reactions to gamma globulin are negligible by comparison, and this has been confirmed (100). Doses of 40 cc have been given intramuscularly to a considerable number of children; and in one investigation, the dose ranged from 20 cc. in infants to 100 cc. in children over 12 years of age (88) Apart from some local discomfort, these large doses were well tolerated.

Perhaps more important is the freedom from homologous serum jaundice The fact that hepatitis might follow the injection of certain lots of human convalescent serum within 2 to 4 months was first pointed out in England (110). Pooled plasma or serum is more apt to transmit this disease than whole blood because the pooling process increases the probability of transmission of an infectious agent from one person to another (115). Because of the very large size of the initial plasma pools, it was feared that administration of any of the products of plasma fractionation might frequently be followed by this distressing complication Studies on this point have been most complete with gamma globulin In 1,977 injections of 85 preparations derived from the blood of approximately 300,000 donors, only 1 instance of jaundice was observed within 6 months of injection. In this particular case, 73 other individuals received the same preparation without contracting the disease; it is therefore assumed that this was probably a spontaneous case of infectious hepatitis (105).

Measles. The effectiveness of convalescent serum, adult serum, or a globulin fraction derived from placental blood, in preventing or modifying measles when administered to a susceptible individual in the first few days after exposure has been abundantly documented These preparations all have certain disadvantages from the practical standpoint Convalescent serum is hard to obtain, adult serum must be used in rather large doses, and both carry the risk of homologous serum jaundice Placental extract (immune globulin) has proved very useful, but its principal drawback is the frequency of reactions and its somewhat variable potency

Proof of the effectiveness of gamma globulin, in the prevention

* It seems most likely that these were coincidences, but they are recorded for completeness

of measles, even when used in extremely small doses, was first brought forward by Stokes, Maris, and Gellis (119), and confirmed by Ordman and others (111). Subsequent papers have extended the original observations, on the basis of reports about the use of gamma globulin by numerous physicians in various parts of the United States (104,105), or as a result of independent investigations (86,124). The use of gamma globulin for measles prophylaxis has a widely accepted practice in the United States, now that the American Red Cross has made available surplus globulin to public health agencies for civilian distribution.

Although there will always be good reasons for using an agent for passive protection against measles, it should be stressed that the goal of preventive medicine is obviously the discovery of a satisfactory method of active immunization. Attempts to do this have been made (*121*), but there is no indication that the problem is near solution. Consequently, we are forced to fall back upon having the disease—preferably in mild enough form to escape its discomforts and serious complications, yet sufficiently to develop a lasting resistance to reinfection. Except in interepidemic periods, when the virus may be less virulent, this must be accomplished by the administration of antibody during the interval between exposure and the development of symptoms. For success, the time of initial exposure must be known (this is frequently difficult to determine) and the proper dose of antibody must be administered at the right interval after exposure. The results in any individual will depend upon three principal factors: (*1*) susceptibility of the individual, which certainly varies, perhaps on a hereditary basis; (*2*) amount of antibody injected; (*3*) length of interval between exposure and injection. To what extent strains of measles virus vary in virulence from one epidemic to another and from one part of an epidemic to another is not known, but such variations will obviously affect the results

The results of the use of gamma globulin in large groups of people will depend upon two other factors: degree of exposure, and age of the group. Although Stillerman and Thalhimer (116) showed that once an individual was adequately exposed to measles additional exposures did not increase the likelihood of his contracting the disease, it is very clear that the chance of an individual's being exposed at all depends on the intimacy of his contact with the pri-

mary case. When exposure takes place in poor homes, the rate of attack will be maximal, while following "exposure" to another case in a hospital ward, the attack rate may be quite low. The effect of globulin will obviously appear to be greater in the latter instance if it is given to all contacts Furthermore, if exposure is adequate, the attack rate varies with age. It is low in early infancy and adult life, but averages from 75 to 85 per cent between the ages of 6 months and 12 years (116).

With these considerations in mind, an effort has been made to ascertain the importance of the two variables under the physician's control which determine the outcome of the administration of gamma globulin to a patient recently exposed to measles The results of these studies, based on more than a thousand reports of instances of intimate home exposure to measles of children between 1 and 12 years of age, indicate that the dose of globulin makes more difference in the outcome than the time of its administration, although the latter affects the results to some extent It is recommended that globulin be administered within the first 6 days after exposure, preferably at the time the diagnosis of measles is made in the primary case as a result of the appearance of Koplik's spots or a rash A dose of 0 1 cc of globulin per pound of body weight will prevent the disease in approximately 80 per cent of intimately exposed susceptible children, the remainder developing mild measles, while a dose of 0 02 to 0 025 cc. per pound will result in modified measles in 65 per cent, average measles in 10 per cent, and no disease in the remaining 25 per cent of those exposed (Fig. 10). These dosage recommendations agree very closely with those of Stokes, Maris, and Gellis (119) on the basis of their initial experience with globulin If globulin is administered after 7 days, larger doses will be necessary

The physician, then, has in gamma globulin a safe and standardized agent for the prophylaxis of measles. He may modify or prevent the disease by varying the dose injected, subject to the uncertainties which are bound to creep into any such complex biologic system Which should he do? It is obviously foolish to attempt to prevent all infectious diseases except by active immunization Measles, like many other infectious diseases, is severe during the first 3 years of life, becomes milder in childhood, and then grows increasingly severe with advancing years The time required to have

a disease like measles is better spared in childhood than in adolescence or adult life. Thus the goal should be to modify the disease whenever possible in the hope that permanent immunity will be

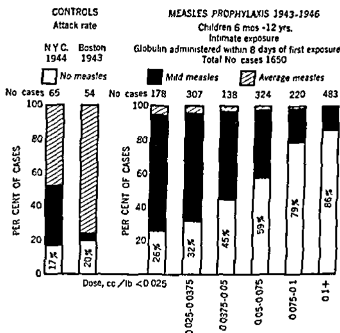


Fig 10 Results of use of different doses of gamma globulin for prophylaxis of measles (Compiled from reports by physicians all over the United States, and from Great Britain and Australia)

established as a result of a mild attack However, there are certain specific conditions under which the disease should probably be prevented. Between 6 months and 3 years, measles is a serious disease as judged from mortality figures; hence it should be prevented in this age group In children with serious chronic illness or with other infections, particularly due to the β-hemolytic Streptococcus, Staphylococcus aureus, Pneumococcus, Hemophilus pertussis, or the tubercle bacillus, or in children exposed to these infections, measles should be prevented, since it appears to lower resistance of the respiratory tract to invasion by these organisms The disease should be prevented in pregnant women, who have been exposed and who

have never had the disease. In addition to these indications, where prevention is in the interest of the individual, there are frequently good indications for preventing the disease for the benefit of a group. Exposures on hospital wards or in institutions, in military personnel, or at times when travel cannot be avoided all may call for prevention, although attenuation may be preferable in certain instances.

The experience with the use of gamma globulin in children's wards has been most gratifying. In 41 outbreaks exposing some 455 children, only 1 case of average measles and 13 cases of mild measles have occurred among 349 children adequately followed (106). In other words, 96 per cent of the children escaped infection. In many hospitals, the ward is no longer quarantined after a case of measles breaks out. Instead each susceptible child is given a preventive dose of globulin. Children entering the ward for the next 3 weeks, who have not had measles, should probably also receive globulin, but it has been our practice not to immunize them unless a secondary case develops among the group originally present on the ward

It is important to know how long a dose of globulin is effective. Even small doses appear to have some modifying effect on the disease if exposure occurs within 3 weeks of injection. The larger preventive dose will exert some effect for 6 to 10 weeks; but if steady protection is desired, it should be repeated every 3 weeks.

Mild measles, following the injection of a modifying dose of gamma globulin, does not differ from that following the use of convalescent serum, normal serum, or placental extract, or from the disease as it may occur spontaneously in a resistant individual or at a time when the virus is of low virulence. The incubation period may be prolonged to as much as 16 to 18 days, chiefly because the prodromal symptoms are of very short duration or absent. Fever may be high and short-lived, of low grade, or absent, but the constitutional symptoms of malaise and prostration are strikingly diminished Catarrhal symptoms are minimal. The rash is frequently quite faint, sparse, and relatively evanescent, while Koplik's spots are seldom seen All grades of illness may be observed, from a disease only slightly milder than the average case of measles to questionable cases without rash but with slight coryza, cough,

fever of 99 F., coming on 2 weeks after exposure. How severe an attack of measles an individual must experience in order to develop permanent immunity is an important problem for future study.

The production of modified measles is justified on two premises: first, that such an attack does produce a lasting immunity in most individuals; and second, that mild measles is less dangerous than severe measles Although there is suggestive evidence that the first of these premises is generally true, careful investigation will be necessary before it can be considered as established Evidence on the second premise is somewhat more convincing, but not absolutely conclusive. The dangers of measles arise from its major complications—secondary bacterial infections and encephalitis. The newer chemotherapeutic drugs have greatly strengthened our hand in dealing with the first, and the second occurs so rarely that it is hard to get significant figures In a small group of cases (approximately 500) given gamma globulin, the incidence of complications was 10 times as high in those who developed severe measles as in those who had a modified attack. In an institution where severe measles and β-streptococcic infection occurred together, the incidence of otitis media was 6 times as high in those with measles who had not had gamma globulin as in the group who developed measles after its administration (111).

Infectious Hepatitis. The importance of infectious hepatitis as one of the major epidemic diseases of mankind has been forcibly brought home by its prevalence in many parts of the world during the last few years On the basis of many older studies, supplemented by investigative work carried on during the war with human volunteers, we have come to recognize infectious hepatitis as a disease which becomes epidemic particularly when there is a break in sanitation, since its principal mode of spread is probably from the feces of one individual with the disease to the mouth of the next susceptible recipient Large epidemics may occur in armies under field conditions. In civilian populations, the disease appears to spread more slowly (112), like poliomyelitis, from individual to individual, at times rather capriciously. As in poliomyelitis, also, protective antibodies against the virus are apparently present in the blood of a majority of adults, few of whom can recall ever having had jaundice.

Stokes and Neefe (122) first demonstrated the effectiveness of

gamma globulin in the prevention and attenuation of this disease. They observed a very extensive outbreak in a summer camp, probably due to contamination of the drinking water from a well so situated that fecal discharges from the initial case of the disease could gain access to it. On the basis that hepatitis had not been observed after the administration of gamma globulin for measles, they felt that antibody might be present, and a portion of the unaffected campers were given an intramuscular injection of 0.15 cc. per pound of body weight. The results yielded conclusive evidence that gamma globulin could in many instances prevent the disease and in others attentuate it, even when administered quite late in the period of incubation. Further studies among troops in Italy (98) and in this country (102) have substantiated this finding. In all cases, using doses ranging from 0.08 cc. per pound to 0.15 cc.

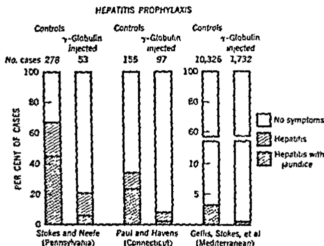


Fig. 11. Gamma globulin in prophylaxis of infectious hepatitis. (Compiled from published reports on use of γ-globulin to control epidemics of the disease.)

per pound, it has been possible to reduce the incidence of clinical jaundice to at least one-fourth its frequency in the control group and to mitigate its severity when it does occur. In two series of cases where careful observation was possible, the use of globulin appeared to reduce the total incidence of probable hepatitis, both

with and without jaundice, to about one-third that in the controls (Fig. 11). Thus, in two outbreaks in the United States and in several large-scale trials in Italy, gamma globulin has been found to produce results in infectious hepatitis similar to those observed in measles. The dosage for preventing either disease is approximately the same, but, unfortunately, little is known of the dose needed to modify infectious hepatitis, and studies to determine this should be carried out, if possible.

The considerations which apply to the use of gamma globulin in the control of measles apply equally to its use in infectious hepatitis. Until methods for active immunization are developed, both infections must be contracted by the great majority of individuals. As with most other infectious diseases, the severity of infectious hepatitis tends to be greater in adult life and its long period of disability is particularly inconvenient at that time. Hence, it may be desirable to contract it during childhood, and gamma globulin should probably not be used in full protective doses except under special conditions—as in pregnant women, in patients who are debilitated, and in groups in which the disease would seriously interfere with essential activities, as among hospital or military personnel. Further difficulties arise in the utilization of gamma globulin *for the control of this disease under the ordinary circumstances of* civilian life. Its mode of spread is not clearly understood, there is no available test for susceptibility, and the proportion of recognizable attacks to inapparent or atypical cases is relatively small. Hence, when a case occurs in a family, we cannot predict with regularity, as we do with measles, how many children will develop the disease nor when their symptoms will occur. Moreover, although the protective dose of gamma globulin appears to be the same for both measles and infectious hepatitis, we do not know whether the modifying dose for measles will be effective in attenuating hepatitis

With hepatitis, as with measles (119), gamma globulin is notably less effective when administered for treatment of the disease, once its symptoms have occurred, than when administered for prophylaxis during the incubation period. Gellis and co-workers (99) treated a considerable group of patients in the early stages of hepatitis in Italy; they were unable to demonstrate any significant *difference* in the course of the disease between the treated and control groups.

The demonstration that epidemics of infectious hepatitis may be controlled with gamma globulin is extremely important from the point of view of public health. It has also added important knowledge to our understanding of the epidemiology of the disease. The fact that gamma globulin from adult blood collected in three different geographic areas of the United States is as effective in the control of the disease in Italy as it is in this country suggests that immunity is fairly universal in our adult population and that the epidemic disease is usually caused by a single immunological strain of virus

Homologous Serum Jaundice. The occurrence of jaundice after an incubation period of 6 weeks to 6 months as an occasional sequel of the injection of even small amounts of human blood, plasma, or serum is a well-established clinical fact (110). There is considerable evidence to suggest that most cases of serum jaundice are not caused by the virus of infectious hepatitis, but by a distinct strain with different properties, i e , long incubation period, presence in the blood for a considerable portion of the incubation period, and lack of infectivity when fed Although the disease produced by these serum jaundice strains is clinically indistinguishable from infectious hepatitis and its pathologic picture is entirely comparable, there does not appear to be cross immunity between the two diseases.

One possible explanation for the fact that the injection of gamma globulin derived from many large plasma pools has not been followed by serum jaundice could be the presence of neutralizing antibodies After the demonstration that infectious hepatitis could be controlled with gamma globulin, Dr Stokes and associates set up several studies to see whether similar results could be obtained with serum jaundice The technic in the different studies has not been identical and the results are conflicting, so that further investigation will be required (93,101).

Other Diseases. There are a number of infectious diseases against which gamma globulin might be expected to have a protective effect These may be divided into two groups: (*1*) those to which the infant appears to have an immunity in the early months of life, presumably due to the passive transfer of antibodies across the placenta from maternal to fetal circulation, and (*2*) those to which the infant has no such immunity. The first group includes:

German measles, measles, mumps, poliomyelitis, scarlet fever, diphtheria, and probably infectious hepatitis; the second: chickenpox, pertussis, epidemic diarrhea of the newborn, and the ordinary bacterial infections. In the first group one might expect antibody derived from pooled adult serum to be effective in the protection of susceptibles, while in the second group one could hardly expect any such result. Clinical studies have born out these expectations fairly well, as is shown in Table I.

TABLE I
Summary of Results with Normal Serum Gamma Globulin in Common Diseases

Disease	Immunity in infancy	Value of globulin	
		Prevention	Treatment
Measles	Present	Effective	Questionable
Infectious hepatitis	Present	Effective	Ineffective
Mumps	Present	Ineffective	Ineffective
Scarlet fever	Present	Under study	Under study
Poliomyelitis	Present	Not known	Ineffective
German measles	Present	Under study	Not known
Chickenpox	Absent	Ineffective	Not known
Infantile diarrhea	Absent	Ineffective	Ineffective
Homologous serum jaundice	Not known	Under study	Not known

From Table I it can be seen that gamma globulin has been proved definitely ineffective in the control of those diseases in group 2 where it has been tried The only exception to the expected findings is in the case of *mumps* in group 1 The results with mumps have been unequivocal We have administered as much as 56 cc. of a gamma globulin solution, with a complement-fixing titer comparable to mumps convalescent serum and representing the antibody from 1,400 cc. of pooled human plasma, to an adult on the sixth day after exposure to mumps without any effect. Enders and Stokes (95) demonstrated conclusively that gamma globulin administered in 10 cc. doses to susceptible children about a week after exposure had no effect on the incidence of the disease. Such unexpected results deserve an explanation, particularly since convalescent serum has been reported effective in passive protection against mumps. There are a number of possible explanations. First, complement-fixing antibody, although significant as an index of immunity, may be distinct from neutralizing antibody, which may be concentrated in a different fraction. Second, apparent immunity to mumps in early life may be due to local tissue factors and not to the presence of

protective antibody in the blood Finally, it is possible that in the pathogenesis of mumps the virus is protected from the action of antibody in some such site as the brain throughout the period of incubation and only enters the circulation very soon after exposure and not again until just before the onset of symptoms If this were the case, antibody present at the time of exposure, as in the case of the infant, might prevent infection, whereas antibody injected a few days after exposure might not be able to reach the virus and might have fallen to too low a titer to be effective at the time of onset of symptoms Clearly, further studies are needed before we shall be in a position to understand immunity in this disease

German measles has always been considered a minor disease, until the recent studies of Australian workers (123) which have been confirmed by a number of other investigators. These indicate that if this infection develops in the first trimester of pregnancy, the infant has a considerable chance of being born with congenital defects, particularly of the eyes or heart (87). Therefore, methods for the protection of pregnant women who have had a known exposure to rubella are urgently needed. That gamma globulin may provide such protection seems possible (117) but has not yet been adequately tested

The control of *poliomyelitis* remains a baffling problem. Adult serums almost universally contain neutralizing antibody to those strains of virus which have been tested Gamma globulin, according to measurements made by Kramer (107) in mice, cotton rats, and monkeys, has a considerably higher titer of protective antibody against the Lansing strain of poliomyelitis virus than convalescent serum The extreme practical difficulty in setting up conclusive experiments to determine whether gamma globulin might be used for the passive protection of children in an epidemic area has hindered studies on this question However, a controlled study of its value in the treatment of cases of preparalytic poliomyelitis was made in the summer of 1944 during a severe epidemic in western New York State by Bahlke and Perkins (88) The results, as anticipated from previous studies on the use of convalescent serum, demonstrated that human antibody has no effect on the outcome of the disease, even when administered in very large doses before the appearance of paralysis

Scarlet fever is a disease for which convalescent serum has been

strongly recommended by certain workers, both as a therapeutic and prophylactic agent. Normal serum gamma globulin contains antitoxin to the erythrogenic toxin of the hemolytic streptococcus in a concentration 3 to 5 times as great as in the usual pool of convalescent serum (Fig. 9). Thus, a dose of 50 cc. of gamma globulin is the equivalent in antitoxin of 150 to 250 cc. of convalescent serum. In a small series of children exposed to the disease at home and given gamma globulin prophylactically, the disease failed to develop, but many more cases will have to be studied before conclusions may be drawn Its therapeutic use in scarlet fever has also been studied by Landon, Jackson, Toomey, Weinstein and others with varying results

Infections, principally those due to common respiratory and intestinal pathogenic bacteria, have been one of the major causes of mortality and morbidity in premature and newborn infants. In an effort to control this, gamma globulin was routinely administered at regular intervals to alternate babies in a premature nursery (125). However, the morbidity was almost identical in the two groups

Studies on the value of gamma globulin in the prevention of respiratory infections in older children and adolescents have not been conclusive as yet (85,120,126). Its use in the prevention and treatment of epidemic diarrhea of the new born has not proved beneficial (103,118).

Gamma Globulin from Convalescent and Hyperimmune Serum. The process of fractionation of plasma makes it possible to concentrate the gamma globulins and the antibody activity associated with them a certain number of times This is limited by the solubility and viscosity of the resulting globulin solution. The antibody titer of the final globulin solution will depend both on the degree to which specific antibody has been purified and on the titer of the plasma pool from which it is derived By using plasma with an elevated antibody titer as starting material, much more potent gamma globulin solutions can be prepared than are possible from pooled normal plasma. A comparison of the antibody levels in different types of plasma and their gamma globulin fraction will be found in Figure 9 Gamma globulin has been prepared from convalescent scarlet fever plasma, mumps convalescent plasma, and also from hyperimmune pertussis plasma

The clinical use of convalescent scarlet fever gamma globulin is under study. Its antitoxin titer is considerably higher than normal serum gamma globulin, and hence it should be particularly useful in those cases where toxemia is very severe and very large doses of antibody are needed. If plasma is collected from convalescents in a particular epidemic due to a single or a few types of hemolytic streptococcus, antibacterial antibodies against these strains should also be present in the globulin The recent work of Rothbard (114) indicates that convalescent plasma should be collected about 6 weeks after the onset of scarlet fever rather than at 3 weeks, as is customary, if the titer of antibacterial antibody is to be at a maximum.

Gellis, McGuinness, and Peters (97) collected mumps convalescent plasma during a severe outbreak of the disease at an Army camp. According to the titer of mumps complement-fixing antibody, the gamma globulin prepared from this material should have been about 8 to 10 times as potent as normal gamma globulin. Lacking methods for measuring protective antibody, we were of course unable to assay its potency accurately. This convalescent mumps gamma globulin was tested in a therapeutic experiment. A series of men entering the hospital on the first day of parotitis were selected for study and every other man was given 20 cc. of mumps convalescent gamma globulin. The incidence of orchitis was about one-fourth as great in the treated group as in their alternate controls. In a second series of cases, every alternate patient was given 50 cc of normal serum gamma globulin. The results showed only slight reduction of the incidence of orchitis in those receiving the injections However, as judged by complement-fixation titers, doses of 160 to 200 cc would have been needed to give a dose of antibody comparable to that administered in the convalescent material

Because of the lack of a suitable therapeutic drug for treatment and because of the difficulty in establishing an active immunity before the age of 8 to 9 months, antibody preparations have come into rather general use for the therapy and prophylaxis of pertussis in small infants Both hyperimmune rabbit serum (90) and hyperimmune human serum (109) have been used. The latter is prepared by immunizing groups of adults who have had pertussis in childhood with H pertussis vaccine, until a high titer of agglutinin is found in their blood. The donors are bled at regular intervals and

their antibody titer maintained by suitably spaced "booster" injections of vaccine. The effectiveness of such human hyperimmune serum in passive protection after exposure is clear cut; its effectiveness in ameliorating the course of the disease when given after the onset of symptoms is less clearly established, but the evidence suggests that it is quite effective when given early (96). Although the volume of the serum to be injected may be considerably reduced by packaging it in the dry state so that it may be reconstituted with less than the original volume of water, the injections of 5 to 10 cc for prophylaxis and 30 to 40 cc for treatment remain rather large for small infants. Such serum has been concentrated by the process of fractionation, and may be obtained in 2 5 cc. vials* which are the equivalent in agglutinin content of a considerably larger volume of unconcentrated serum Lapin (108) has reported very satisfactory results with this material

The preparation of gamma globulin from convalescent and hyperimmune serum has many possibilities in respect to public health.† Convalescent serums are frequently difficult to obtain, and carry the risk of homologous serum jaundice when properly preserved by freezing and drying. By collection when available, storage in the frozen state, and fractionation when a sufficiently large amount of material is accumulated, the antibody may be processed to a stable powder which can be kept until needed during an epidemic, when it may be put into solution for use The risk of jaundice would appear to be diminished, according to the experience with gamma globulin so far Moreover, all the other products of plasma fractionation may be obtained from convalescent plasma as well as from normal plasma, so that a considerable economy of valuable products is achieved The production and concentration of human hyperimmune serum has only been applied to pertussis as yet, but there are other obvious possibilities.

Enzyme Digested Gamma Globulin. Methods for the concentration and reduction in antigenicity of antibodies derived from animal serums by enzymic digestion have been applied to human serum gamma globulin by the group working with Dr. J. W. Williams at the University of Wisconsin (91,92,113) By selecting the

* Marketed by Cutter Laboratories, Berkeley, Calif, under the name *Hypertussis*

† Successful use of gamma globulin from convalescent rubella plasma to protect pregnant women from German measles has just been reported from Australia and Holland (196)

proper conditions and enzymes, preparations may be obtained in which most of the antibody is present as half-molecules or quarter-molecules. Such antibody globulin is less viscous and more soluble, making possible more concentrated solutions. The effect of such changes in antibody size on their distribution in the body or their ability to penetrate and be absorbed is not known as yet

Results of Theoretical Interest. The many studies which have been made with normal human serum gamma globulin have considerably extended our understanding of human immunity. Failure of normal human antibodies to prevent mumps and equivocal results in serum jaundice have pointed to gaps in our knowledge of these two diseases which may well be filled in when studies arising from these failures are completed The combined laboratory and field studies with human serum gamma globulin prepared from blood collected in different parts of this country provide the first really comprehensive survey of the immunity status of the population of the United States If such studies continue as long as plasma is fractionated, the appearance of major epidemics of influenza, increases in the diphtheria carrier rate, and similar epidemiologic changes should be reflected in the antibody levels of the population in different sections of the country, and may be charted by public health authorities for a permanent record of great value The fact that gamma globulin from adults in the United States, a very small percentage of whom could give a history of jaundice, protected troops against epidemic hepatitis in Italy is our best proof that this disease is a very widespread one which has immunized large sections of our population by unrecognized inapparent infection Even the potency of gamma globulin against measles should be studied at regular intervals No one can predict today what will be the status of measles antibody in a population which has generally experienced a very mild form of measles as a result of the routine use of gamma globulin The fact that regular injections of normal serum gamma globulin do not significantly increase the resistance of premature infants to infection is another bit of evidence to support the hypothesis that their susceptibility to infection is less the result of a deficiency of antibodies than the consequence of a general immaturity of the adaptive mechanisms. Thus, this fraction which has made such practical contributions to the control of infectious disease has also served as a tool for investigation of fundamental problems in human immunity

HUMAN SERUM ALBUMIN

Chemistry of Serum Albumin

The protein precipitated in Fraction V represents approximately one-half of the total plasma proteins. It contains 97 to 98 per cent albumin and from 2 to 3 per cent alpha globulins. Both bovine and human serum albumins have been crystallized from Fraction V of the corresponding plasma by Cohn and Hughes, but in the case of human albumin there is no practical reason for carrying the process of purification to this extent, particularly since it entails a considerable loss of material.

Albumin is the most soluble, most stable, and most osmotically active of the plasma proteins (1–4); because of the relative symmetry of the albumin molecule (Fig. 6), its solutions have a low viscosity (33). This combination of properties was responsible for the development, in response to the urgent needs of the Navy, of concentrated normal human serum albumin as a compact, convenient, and effective blood substitute for emergency use (162,171). Because of its solubility and stability, it is possible to distribute albumin in concentrated solution rather than in the dry state—an important military consideration, since the space required for the bottle of diluent and the time required for reconstitution are eliminated Moreover, a solution which can be kept in a military supply depot, in a doctor's bag, or on the shelf of an accident room, even in hot climates, without marked deterioration has great practical advantages.

For obvious military reasons, a great deal of attention has been devoted to the problem of thermal stability of albumin solutions It is maximal at pH 6 8, and may be increased by raising the concentration of sodium chloride in the diluent. The improved stability resulting from an increase in sodium chloride from isotonic (0 15 molar) to twice isotonic (0 3 molar) concentration was sufficient to justify the specification of 0 3 molar (1.8 per cent) sodium chloride solution as the diluent to be used in the standard Army–Navy albumin package (175). The salt content of the solution was no disadvantage for the treatment of shock, but with the application of albumin to the treatment of edematous states, particularly in renal disease, there was good reason for reducing the sodium in the diluent to the lowest possible figure if it could be done without loss of

stability. The discovery (132,133) that nonpolar anions markedly increased the thermal stability of serum albumin made this possible. The sodium content of a 25 per cent albumin solution containing a suitable stabilizer can be reduced to approximately 0.3 Gm. per cubic centimeter. Table II shows the amounts of sodium in osmotically equivalent volumes of plasma protein solutions

TABLE II
Amounts of Sodium in Osmotically Equivalent Quantities of Plasma Protein Solutions

Solution	Protein, Gm.	Volume, Cc	Sodium Gm
Normal human plasma, citrated	30	525	2.1
Normal human serum albumin (original standard solution)	25	100	0.97
Normal human serum albumin (low-salt or salt-poor)	25	100	0.3

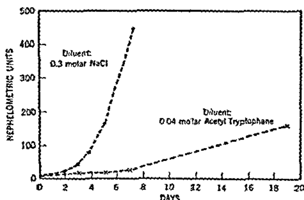


Fig 12 Comparison of thermal stability (heating at 57 C) of a single preparation of normal serum albumin (HA 171), when dissolved in 0.03 molar (1.9%) sodium chloride and in 0.04 molar acetyl tryptophane There is a marked increase in stability when the latter is used as a stabilizing agent.

Various nonpolar anions which appear to form complexes with albumin (134,136,137) have been proposed for this stabilization, among them phenyl acetate, caprylate, and mandelate, acetyl phenylalaninate, and acetyl tryptophanate (Fig 12) Caprylate

appears to be the most effective but is more toxic (164), whereas acetyl phenylalaninate and acetyl tryptophanate, as derivatives of natural amino acids, are well tolerated There is a physiologic argument in favor of the choice of acetyl tryptophane to increase thermal stability, since Hegsted, Hay, and Stare (154) showed that serum albumin was an incomplete protein in the diet of growing rats unless supplemented with *l*-tryptophane, an amino acid in which albumin is deficient, and *l*-isoleucine, in which all plasma proteins are deficient

By contrast, human serum albumin was found capable of maintaining the nitrogen balance in adult dogs when it supplied the sole protein and only 6 per cent of the calories of the diet Davidson and associates (144) have studied the nutritional adequacy of serum albumin in man when administered by mouth and by vein, with and without added acetyl tryptophane, as the sole source of protein in the diet. Despite the inherent technical difficulties of such an experiment, their findings suggest that serum albumin, which is handled like any protein when fed, is much more slowly metabolized when administered intraveneously, as shown by a very gradual rise in nitrogen excretion and a selective rise in total circulating plasma albumin during administration. They believe that as serum albumin is metabolized it is broken down into its constituent amino acids, rather than absorbed into the cells as such Furthermore, from their evidence that intravenously administered albumin is ultimately broken down and that orally administered albumin will maintain nitrogen equilibrium in normal adults, they infer that the nitrogen of plasma albumin is available for use when needed More insight into the fate of intravenously administered plasma protein is being provided by the technic developed by Albright, Forbes, and Reifenstein (128) for determining the amounts of injected protein burned for energy purposes, converted to protoplasm, and retained unchanged.

Because it is a derivative of a natural amino acid, essentially nontoxic, and possibly of nutritional importance, 0 04 molar acetyl-*dl*-tryptophane was authorized for routine use as a stabilizing agent for serum albumin, as soon as the safety of such preparations was proved in the clinic. This amount will increase thermal stability to such an extent that a 25 per cent solution of serum albumin containing only 0 3 Gm. sodium per hundred cubic centimeters may be

heated at 60 C. for 10 hours as a part of the standard processing procedure. Even after this treatment, a serum albumin solution of low salt content ("salt-poor albumin") (176), when properly prepared, has a stability comparable to that of the former standard Army-Navy albumin solution containing 0.3 molar sodium chloride.

The period of 10 hours at 60 C. was fixed as the most practicable period of heating which might be expected to destroy the virus of hepatitis should it be present in the solution, although the thermal death time of this agent was not then known. It was subsequently demonstrated that this period and temperature were adequate to inactivate the virus of serum hepatitis (149). By including heating of albumin in the final containers as a step in the process of production, it should be possible to destroy the virus of hepatitis as well as most bacteria. The only exceptions are bacteria present as spores, and these should be detected if the heating period is followed by a period of incubation. This procedure makes it possible to eliminate the mercurial preservative formerly used in albumin solutions, and thus renders the use of albumin in large amounts safer.

In addition to its thermal stability in properly prepared solutions, the outstanding chemical properties of albumin are its solubility and low viscosity. These permit the ready preparation of a 25 per cent solution which may be sterilized by Seitz filtration and easily injected with ordinary intravenous needles. Low viscosity is important in therapy for the injection of concentrated albumin should not increase but actually reduce the viscosity of the blood, since fluid is drawn into the circulation by its osmotic effect. Thus present specifications for "salt-poor" albumin, i.e., of low salt content, call for a 25 per cent solution in a diluent at pH 6.8, containing approximately 0.3 Gm. of sodium per hundred cubic centimeters and acetyl-dl-tryptophane in 0.04 molar concentration. The solution contains no preservative, and is heated for 10 hours at 60 C. in the final container (176).

The relatively small size of the albumin molecule and its high net charge combine to make it the most osmotically effective of the plasma proteins. Although all of the plasma proteins contribute to the colloid osmotic pressure of plasma, it is chiefly due to the albumins and to the alpha and beta globulins of Fraction IV-4 which have a smaller average molecular weight and a higher net charge

than most of the other globulins (33). Unfortunately, these alpha and beta globulins are less stable than albumin, and must be separated from it in Fraction IV-4 if albumin is to be packaged in solution ready for use. Studies on the comparative osmotic pressure of plasma and serum albumin by Scatchard, Batchelder, and Brown (177) have demonstrated that the molecular weight of the latter is approximately 69,000, compared with an average molecular weight for whole plasma proteins of about 90,000. As a result of their studies, these authors concluded that, in a patient with a normal plasma protein concentration (assumed to be 7 Gm/100 cc.), each gram of albumin should be equivalent in osmotic effect to 1.2 Gm of whole plasma proteins and should hold 18 cc. of fluid in the blood stream. Thus, the increase in blood volume resulting from injection of each gram of albumin should be equivalent to that resulting from the injection of 18 cc. of plasma as it circulates in the body, or 20 cc. of the usual pooled citrated plasma. On this basis, the 25 Gm of serum albumin in 100 cc. of diluent which constitute the contents of the standard Army–Navy package of normal human serum albumin are osmotically equivalent to approximately 500 cc of citrated plasma.

Clinical Use of Serum Albumin

Physiologic Responses to Albumin Administration. Since serum albumin was developed as a blood substitute for the treatment of shock, the first clinical studies were aimed at defining the response to a given dose of albumin. The effect of albumin on blood volume was studied experimentally in man by Heyl, Gibson, and Janeway (155,156). Subjects in the basal state whose blood volumes were rapidly depleted by venesection were given concentrated albumin, and the resulting increase in plasma volume was carefully measured. In these experiments, based on procedures developed for the study of shock in man by Stead, Gibson, and Ebert (143,181) under the stimulus of Dr. Soma Weiss, the exact amount of blood lost could be accurately measured and further losses of fluid, which confuse studies in clinical cases of traumatic shock, were obviated. These studies demonstrated: (*1*) that concentrated albumin solution produced a rapid increase in the plasma volume comparable to the one observed after infusion of reconstituted dry plasma,

(2) that concentrated bovine and human serum albumin produced similar osmotic effects; (3) that concentrated albumin was as effective in moderately dehydrated subjects as in those who had received excessive amounts of water and salt in preparation for the experiment; (4) that concentrated serum albumin produced an average increase in plasma volume of approximately 17 cc. per gram of albumin injected, a figure which agrees remarkably well with the expected figure of 18 cc. per gram derived from the osmotic pressure measurements of Scatchard *et al.* (Fig. 13). Thus the selection of

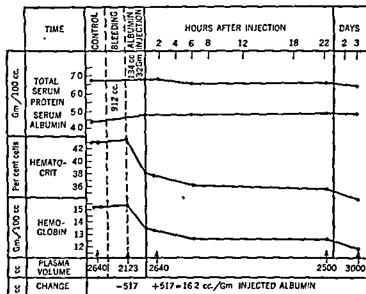


Fig 13 Osmotic activity of normal serum albumin after experimental blood loss A normal subject was bled 912 cc and immediately given 32 Gm (134 cc) of concentrated human serum albumin Chart shows changes in plasma volume, hemoglobin, protein concentrations, and in hematocrit reading (155,156).

25 Gm in 100 cc. as a unit to correspond with 500 cc. of plasma was further justified

The immediate increase in plasma volume following an injection of concentrated human serum albumin, as shown by a fall in hemo-

globin and hematocrit, occurs whenever albumin is administered rapidly. Whereas in subjects with an initially diminished blood volume the hemodilution is maintained, it tends to disappear after the injection of albumin into a patient with a normal blood volume, presumably as excess plasma is removed from the circulation (104). Thus, the extent of this plasma volume increase will depend on the rate of injection and the rate of removal of excess plasma. Because of low viscosity and low sodium content, albumin puts a minimum load on the circulation, but its powerful osmotic effect must not be forgotten when it is administered to patients with severe hypertension or congestive failure. Thorn and colleagues (185) concluded that an injection rate of 10 Gm. per hour could be readily tolerated by most adult patients; they maintained such a rate by diluting 50 Gm. of albumin with 300 cc. of 10 per cent dextrose in water, yielding a solution of 10 per cent albumin in 6 per cent dextrose solution. In actual practice, however, albumin has generally been administered directly as the 25 per cent solution, or mixed with whatever parenteral fluids were being administered at the time.

The ultimate fate of the injected albumin is less clear. The occurrence of albuminuria appears to depend mainly on the state of the kidney; albumin administration may be followed by an almost quantitative increase in protein excretion in nephrotic patients. In normal patients no albumin ordinarily appears in the urine, unless the serum albumin concentration is pushed above the normal level, suggesting that there is a renal threshold for albumin (see reference 188). In patients with chronic hypoproteinemia, although the extra nitrogen injected as albumin is conserved, only a fraction of the protein administered can be accounted for by an increase in total circulating albumin (144,160). Albright and co-workers (128) are investigating the fate of intravenously injected plasma proteins. These studies should ultimately shed some light on the extent to which plasma proteins are burned for energy purposes in the formation of tissue protein, or retained unchanged. Eckhardt's studies suggest that albumin is not rapidly metabolized unless fed (144). Thus, much more needs to be done before any generalization can be made as to the fate of albumin after injection in different types of patients. The following statements are justified by the evidence now available: (*1*) Patients with proteinuria show increased excretion

after albumin injection (*2*) In chronic hypoproteinemia, several times as much albumin must be administered as would be calculated to restore the total amount of circulating albumin to normal. (*3*) Whereas the feeding of albumin to a hypoproteinemic patient may result in an increased output of urinary nonprotein nitrogen, this does not usually occur when it is injected, at least until several days have elapsed.

Safety. Although the osmotic potency of albumin, and hence its efficacy in sustaining the blood volume, could be safely assumed on the basis of the early experimental and clinical studies, the safety of a product of this sort can only be established after extensive experience Each lot of albumin prepared throughout the Red Cross Army–Navy program was tested in patients under observation in civilian and Naval hospitals before its release to the Armed Forces (160) Only tentative conclusions can be drawn from this experience of 1,915 injections involving 600 patients, for the use under field conditions of those preparations of albumin which were released yielded no statistical data, but only the general impression that it was convenient to use and gave a few reactions These may be classified as immediate anaphylactoid, pyrogenic, hemodynamic, displacement of serum globulins, toxic effects of continued large doses and homologous serum jaundice

Immediate Anaphylactoid Reaction This type of reaction is extremely rare, many patients who have urticaria or repeated febrile episodes when given plasma have had no difficulty with albumin. An occasional patient, however, has had urticaria

Pyrogenic Reactions Nausea, headache, and fever with or without chills, 30 to 90 minutes after injection, were by far the commonest symptoms, they occurred in 4 per cent of test injections during the Army–Navy program Since pyrogenic lots were rejected in the Army–Navy program, the incidence of reactions with released material should have been considerably lower This type of reaction is somewhat less frequent in present tests on albumin prepared from surplus dry plasma by the American Red Cross.

Hemodynamic Reactions Such reactions, due not to toxic properties but to the physiologic activity of albumin in improperly selected cases, have been occasionally observed Pulmonary edema has developed in hypertensive nephrotic children given large doses of albumin for diuretic purposes In a few elderly patients, the in-

creased circulatory activity following albumin administration may have dislodged thrombi and precipitated pulmonary embolism, and in a small number of patients with esophageal varices, hemorrhage has followed the administration of large doses of albumin

Displacement of Serum Globulins. The massive replacement of large amounts of lost plasma with serum albumin in the treatment of severe burns might be expected to and did result in a dangerous prolongation of the prothrombin time in one patient given nothing but albumin for the first 24 hours after the burn. This was readily rectified with plasma (160). This type of reaction has only been observed in cases with rapid loss of plasma and massive replacement with albumin.

Toxic Effects of Continued Large Doses Patients receiving large doses of albumin daily for weeks, as in nephrosis, have been carefully observed for toxic effects which might be attributed to the albumin, the preservative (present in the original standard solution), or other substances, such as the stabilizer present in the diluent. No morphologic evidence of abnormal storage has been observed at autopsy in patients receiving large doses (160,185). The only point which has not yet been settled is whether the massive proteinuria induced in nephrotics has any permanent deleterious effects on the kidney. This is now being carefully studied The evidence for the comparative safety of albumin, when properly used, seems overwhelming on all other counts

Homologous Serum Jaundice. Adequate data could only be obtained in a small series. 1,077 injections in 72 patients followed for at least 3 months One case of jaundice occurred 3 weeks after a short course of albumin injections, but this was exactly 3 months after a previous course of plasma transfusions (161). This only suggests that albumin, as processed prior to the introduction of the 10 hours of heating at 60 C., was relatively free from the risk of inducing hepatitis Experimental proof that such a heating step will inactivate the virus has been obtained (149), and data on albumin processed in this way are being collected at present.

Use in Shock. Because albumin was developed for use as a blood substitute, studies during the war were aimed at its evaluation for this purpose, but even these were curtailed because of the urgent need for material. A moderate amount of experimental work in animals, carried out chiefly with crystallized bovine serum al-

bumin, supplemented clinical studies. Dunphy and Gibson (142) compared bovine serum albumin, both concentrated (25 per cent) and dilute (5 per cent), with dog plasma in the treatment of burn shock in dogs Their experiments suggest that crystallized bovine serum albumin is superior to bovine Fraction V for such studies in animals. This is a point worth stressing, for the crystallized product has been freed of most of the 3 to 5 per cent of globulin impurity in Fraction V; this fraction may be somewhat toxic, since whole bovine plasma is very toxic. In their dogs with severe burns, dog plasma or dog plasma supplemented with bovine albumin sustained the circulation well Concentrated albumin alone was quite effective, but microscopic examination of the tissues showed greater damage than in the case of dogs receiving either dilute albumin or plasma

Fine and associates have used bovine albumin most extensively in experimental studies on shock in dogs. In severe tourniquet shock, they were able to demonstrate the need for supplemental fluids if the osmotic effect of concentrated albumin was to be obtained When saline was given, either by vein or by duodenal tube, 25 per cent albumin appeared to be nearly as satisfactory a replacement fluid as plasma (147) Fine (148) and others (131) have used crystallized bovine albumin as an experimental tool in studying experimental shock Human serum albumin has likewise been used in animal experiments (169)

Normal human serum albumin was accepted for the Armed Forces on the basis of a series of reports gathered from a number of cooperating clinics, which demonstrated that its administration to patients in shock from hemorrhage, trauma, operations, and burns was usually followed by hemodilution, rise in blood pressure, and evidences of clinical improvement (160,190) More detailed hemodynamic measurements were subsequently made by two teams, working under contracts with the Office of Scientific Research and Development, one in Atlanta under Drs. Stead and Warren (182,187) and the other in New York City under Drs Richards and Cournand (138,166). Their detailed observations on patients admitted in severe shock have provided many important data on changes in the circulation in shock of various types, and on the responses to various replacement fluids Furthermore, their cooperation with the chemical group in Boston made it possible to

test critically each change in specifications for albumin before allowing this change to take place in material prepared under Navy contract. Thus, their studies demonstrated that in shock patients "salt-poor" albumin was as effective as that containing 0.3 molar sodium chloride and permitted the change to "salt-poor" albumin as a solution adaptable to the treatment of either shock or hypoproteinemia.

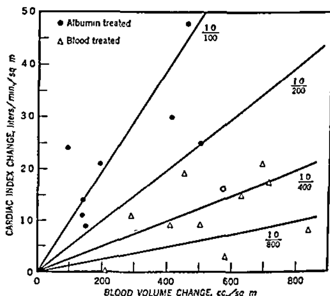


Fig 14 Comparison of increase in cardiac output (cardiac index) in relation to increase in blood volume in patients with traumatic shock treated with human serum albumin or with whole blood (138)

The hemodynamic studies of these two groups confirmed the initial clinical evaluation, and provided convincing proof of the effectiveness of albumin in terms of increased cardiac output. Cournand, Richards, and their associates (138) compared saline as a simple crystalloid solution, albumin as an example of a plasma protein solution, and whole blood, in their hemodynamic effects in shock They pointed out very clearly that the first was ineffective and the second effective in compensating for the diminished *blood volume*, but not in restoring the oxygen-carrying power of the blood. Consequently, a greater increase in cardiac output was required to meet

the demands of the tissues for oxygen after albumin than after whole blood, since injection of albumin in shock substituted a state of compensated anemia for one of uncompensated circulatory collapse (Fig. 14). This work, done in 1943, clearly indicated the need for whole blood, which finally materialized at the fronts with the improvement in air transport, the development of satisfactory blood preservative solutions, and the provision of adequate refrigeration in transit.

Another point of considerable physiologic interest was their demonstration of the relation between blood viscosity and blood pressure To quote from one of their papers (166, p 451):

> "[The] results showed clearly that changes in mean arterial pressure are not a reliable index of changes in cardiac output and that although cardiac output and intra-auricular pressure (venous return) attain normal levels after the injection of concentrated albumin solution, the blood pressure may remain subnormal. It was also pointed out that with hemodilution there would be a decrease in blood viscosity This may account, at least in part, for failure of the blood pressure to rise to accepted normal levels Further evidence that changes in viscosity play an important role in the variation of peripheral resistance was obtained in a study on the intravenous use of gelatin solution of high viscosity in the treatment of shock In this latter study, the increase in blood pressure was relatively greater than the increase in cardiac output"

In the meantime, a comparison between the effectiveness of concentrated albumin and isotonic plasma in the treatment of battle casualties was made in the Mediterranean Theater (135). This indicated that albumin was only about half as effective as it should have been judging from its osmotic equivalence to plasma. Additional fluids were not administered to any of the patients to determine whether this discrepancy was due to this factor. Following this report, a re-evaluation of albumin was made in this country under the direction of Dr Richards, with the cooperation of several groups of investigators (Drs. Stead and Warren, Drs. Cournand and Lowell, Dr. Evans, and Dr. Rhoads). Their observations are in general accord with the previous studies (159). Marked variations in response, as judged by increase in blood volume, were observed between individual cases. A compilation of all the published figures, however, seems to indicate quite clearly that the response in severe shock is slighter, and that additional saline should be given with albumin if the maximum benefit of its osmotic pressure is to be obtained (Table III). This was predicted by the Blood Substitutes

Subcommittee of the National Research Council at the start, and a warning to that effect was etched on the albumin container (171). It could be inferred from the experimental results of Fine and his colleagues (147).

TABLE III*

Osmotic Effect of Concentrated Albumin, as shown by Plasma Volume Measurements within One and One-Half Hours of Albumin Injection

Type of case	Number of cases	Plasma volume increase, cc./Gm. injected albumin	
		Spread	Average
Calculated from osmotic pressure measurements (177)	—	—	18
Experimental hemorrhage in man (155)	11	13.2-24.1	17.4
Clinical shock (no additional saline or small amount) (138,166,187,182)	63	0-31.7	11.7
Clinical shock (additional saline)	20	7-29	17.9

* Revised from Janeway (159).

The place of albumin in the treatment of shock has been very clearly summarized by Stead and associates (182, p. 574):

"Neither plasma nor albumin is a substitute for whole blood. Albumin nevertheless is an extremely useful substitute for plasma. From the standpoint of speed and convenience of administration, convenient packaging, small bulk, stability under varying temperatures and absence of bacterial contamination, concentrated albumin is ideal. In civilian practice, where whole blood and plasma are readily available, albumin may not be used extensively in the treatment of shock, but under the conditions of war, concentrated albumin has many advantages. It is possible that the danger of hepatitis resulting from transfusion of blood and plasma can be avoided by using albumin."

Use in Hypoproteinemia and Edema. *The availability of* serum albumin as a concentrated source of plasma protein with a low sodium content, safe for injection in large quantities, has naturally aroused great interest in its potentialities for the treatment of patients with edema and hypoproteinemia. The importance of hypoproteinemia, or, actually, of lowered colloid osmotic pressure of the plasma, as a factor in the development of edema has been deduced from the results of plasmapheresis experiments and clinical observations in patients with certain diseases in which hypoalbuminemia is a prominent feature. By the use of serum albumin, it is possible to elevate the colloid osmotic pressure rapidly and to subject the oncotic theory of edema formation to closer scrutiny.

It is hardly necessary to point out that a true deficit in circulating albumin may be masked by a diminished blood volume or by hyperglobulinemia if only simple measurements of the total plasma protein concentration are made. Work by Govaerts (151,152) on

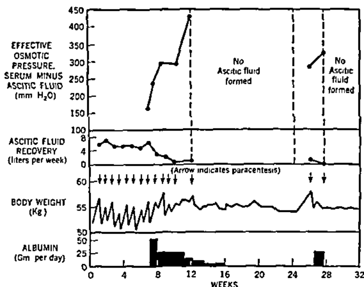


Fig 15 Effect of normal serum albumin in a case of severe cirrhosis of the liver with established ascites Note fall in rate of accumulation of ascitic fluid, with rise in effective colloid osmotic pressure (colloid osmotic pressure of serum minus the colloid osmotic pressure of the ascitic fluid) (courtesy Drs. S H Armstrong, Jr, K Emerson, Jr, and S T Gibson).

famine edema has shown that diminution in colloid osmotic pressure may be greater than the fall in serum protein level, because of the selective reduction in albumin. Armstrong (129) has discussed the use of serum albumin in the study of disturbed osmotic relationships in hypoproteinemia, with results that suggest the importance of other factors beside low oncotic pressure.

Our concern here is to summarize briefly the limited clinical experience with the use of serum albumin for therapeutic purposes. This experience is limited only because until recently there was little albumin available for this type of study.

Therapeutic Use of Serum Albumin.

In Liver Cirrhosis. A limited number of patients with cirrhosis of the liver have been studied. That the level of serum albumin can be rapidly elevated to normal by the administration of 25 to 100

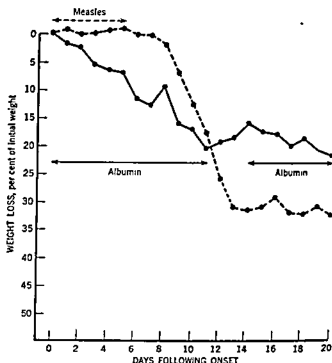


Fig 16 Comparison between the rapid weight loss of a spontaneously occurring diuresis after an infection (measles) and the gradual weight loss during low-salt serum albumin administration in a 3 year old child with the nephrotic syndrome Note rise in the weight curve from the tenth to the fourteenth days, when the injections of albumin were stopped, and resumption of the fall in weight when injections were resumed (158)

Gm per day is clear In patients with long-standing cirrhosis and an inability to maintain their albumin level on a diet rich in protein and calories, the level declines when albumin administration is discontinued. Whereas the administration of albumin will usually result in the disappearance of edema, its effect on ascites is less dra-

matic (160,184). However, Gibson (150), Armstrong (130), and Kunkel and co-workers (165) suggest that the formation of ascites can be stopped in some cases, at least by the continued administration of large amounts of albumin over a considerable period of time (Fig. 15). On the other hand, in three severe cases with prolonged ascites studied by Patek (172), the restoration of a normal serum albumin level had no effect because the rise in serum albumin concentration was paralleled by an increase in its concentration in the ascitic fluid The extent to which the maintenance of a normal serum albumin level will effect the ultimate course of the disease has not been determined. However, some patients can certainly be made more comfortable and kept ambulatory. Further studies are clearly needed before the exact indications, dosage, and regime for the use of albumin in the treatment of cirrhosis of the liver can be delineated.

Disease of the liver does provide the most rational opportunity for the use of serum albumin, since its specific replacement eliminates one of the major disturbances resulting from the inability of the liver to synthesize plasma proteins. In a few instances of acute or subacute hepatitis, serum albumin has been useful as supportive therapy or to accelerate the slow restitution of normal osmotic relationships which may take place with dietary therapy alone (165). However, the rapid spontaneous changes which may take place in such patients call for caution in interpreting results.

In the Nephrotic Syndrome. Salt-poor albumin would be an ideal diuretic agent in nephrosis, were it not for the fact that it is excreted so rapidly in the urine of such patients. Nevertheless, in many instances, small but steady losses of weight are achieved with daily injection of 12 5 Gm (in infants) to 50 to 75 Gm. (in adults) (158,185). The weight loss and diuretic response are slow, compared to the rapid changes during a spontaneous diuresis, such as occurs after acute infections (Fig. 16). Luetscher (167) demonstrated the rapid loss of albumin in the urine after an injection of albumin (Fig. 17). Thorn and co-workers (185) showed that it is very difficult to elevate the serum protein level significantly in these patients, although an expansion of plasma volume may make an increase in circulating albumin.

Two factors must be carefully weighed before recommending the use of albumin in nephrosis; first, the expense in terms of either

blood or money; second, the possibility of damage to the kidney. Albumin prepared from the bloods of 100 to 200 donors is needed to rid a single patient of edema. At commercial prices, its use in the

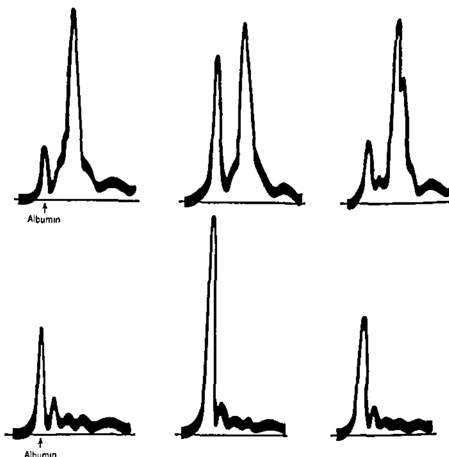


Fig 17 Electrophoretic Schlieren diagrams of serum (above) and urine (below) from a patient with the nephrotic syndrome before (left), 1/2 hour after (center), and 48 hours after (right) intravenous injection of 25 Gm of normal human serum albumin These diagrams show the transient increase in serum albumin and the prompt increase in its excretion in the urine (167)

enormous doses necessary to get temporary benefit is questionable. The matter of damage to the kidney is a more serious consideration The massive proteinuria which follows the injection of albumin may

conceivably be harmful. This point is now under study in several clinics. Meanwhile, physicians should exercise considerable caution in the use of albumin. Encouraging results reported from one clinic (139) may well be due to a number of other measures used in addition to albumin.

Nutritional Edema. Edema is frequently observed in both medical and surgical patients, in whom inadequate food, infections, operations, disturbances of absorption, and protein loss combine in varying proportions to cause hypoproteinemia. Here again experience is limited, but what there is indicates that the use of albumin has great possibilities, particularly for those patients in whom rapid relief of edema is important, as in preoperative preparation (104, 145,160,170,183).

In Other Conditions. There are many other types of edema in which studies should be carried out, for example, cardiac edema, where poor dietary intake and congestion of the liver may play a role in the hypoproteinemia so frequently seen The influence of injections of concentrated serum albumin on local edema, such as cerebral edema resulting from trauma or operations, should also be evaluated Albright *et al* (128) have made the interesting observation that a prolonged positive calcium balance could be induced in a patient with osteoporosis when the serum protein was raised to supernormal levels by administration of large doses of albumin. The relation of such findings to the known binding of calcium by serum albumin is of great interest

Through the American Red Cross, limited supplies of albumin for research purposes have been supplied to many of the University clinics. It is hoped that the potential uses of serum albumin in civilian medicine may be critically studied for the benefit of the profession, who may have access to large quantities of this important blood derivative in the future

BOVINE SERUM ALBUMIN

The original studies on plasma fractionation began with bovine plasma and were continued in collaboration with the Armour Laboratories until it became clear that crystallized bovine serum albumin could not safely be used as a blood substitute on a large scale (157,180). Actually, it was not needed because of the mag-

nificent response of the American people to the call of the Red Cross for blood donors; nevertheless, the development of purified fractions of bovine plasma has made possible a number of important theoretic and practical investigations.

Crystallized bovine serum albumin has been a very useful tool in physiologic research on shock and the factors controlling the distribution of fluid between the capillaries and the tissues (131,142,-147,148,178,191). It has provided the chemist with a large supply of highly purified protein for studies on osmotic pressure (177), on the modification of antigenicity (168,173,189), and on interactions of protein with various organic compounds, such as the sulfonamides (163), and with simple salts (174). Crystallized bovine serum albumin and purified bovine serum gamma globulin have been used as tools for the study of the pathologic and serologic sequences in experimental hypersensitivity in animals (153).

Bovine serum albumin, because of its ability to combine with fatty acids in a medium, has been utilized for the rapid cultivation of the tubercle bacillus (140,141). It has been shown to bring out anti-Rh agglutinins in so-called "blocking" serums if it is used as the medium for suspension of the test red cells (76), a general principle which is rapidly finding wider application in serology. In studies on malaria, flotation methods with mixtures of blood and bovine albumin made it possible to separate parasitized red cells from noninfected cells (146), thus effecting great concentration of the parasites The same principle has been applied to the separation of various types of white blood corpuscles (186). Bovine albumin has likewise been utilized in studies of the density of influenza virus (179)

Summary

Since the introduction of large-scale plasma fractionation methods, a number of useful blood derivatives have been developed and a sizable body of knowledge has been accumulated concerning the "anatomy and physiology" of the plasma proteins.

From the therapeutic standpoint fibrinogen and thrombin, fibrin foams, fibrin films, and fibrinogen plastics, in their own right and as forerunners of similar products derived from synthetic and other natural sources, have led to important advances in surgical technic Fraction I has been established as a valuable agent for the control

of the clotting defect in hemophilia Normal serum gamma globulin has been proved effective in the control of measles and infectious hepatitis, while similar gamma globulins derived from convalescent mumps and hyperimmune pertussis serums have been shown to have specific value in those two diseases Potent, readily standardized, blood grouping and Rh typing reagents can be produced from blood of the proper groups Serum albumin, useful as an emergency blood substitute for the therapy of shock, has far greater promise as an aid in the mobilization of water in edematous patients and for support of patients with serious hepatic insufficiency By the use of the red cells for anemic patients and the various plasma fractionation products for their specific indications, the blood of a single donor may contribute to the treatment of many patients This economy in the use of blood is accompanied by increased safety, as far as the hazards of homologous serum jaundice and the more common pyrogenic and anaphylactoid reactions are concerned.

Equally important are the contributions which these products have made to physiologic investigation. Fundamental knowledge concerning the coagulation mechanism, hypersensitivity and immunity, the transport of iron and many other substances in the blood, the mechanism of Rh sensitization, the growth requirements of the tubercle bacillus and of the malarial parasite, and the control of water balance in the body has already been obtained. Improved methods for the purification of biologic materials, such as enzymes, hormones, toxoids, and viruses, have developed from the principles on which the fractionation methods are based

Acknowledgment

The author would like to acknowledge his personal debt to the many colleagues with whom he has had the pleasure of collaborating during the past

assistance of their technical personnel For what these many colleagues have taught him, as well as for the personal pleasure of his association with them, the author is deeply grateful. Thanks are also due to the Misses Marion Greene, Mary Augustine, and Muriel Y Gratton, without whose help the manuscript would never have gone to press, and to Miss Virginia Poole for invaluable editorial and technical assistance.

References

Introduction

1 Cohn, E. J.: The properties and functions of the plasma proteins, with a consideration of methods for their separation and purification Chem Rev. *28*, 395, 1941.
2. Cohn, E. J., Oncley, J L, Strong, L. E, Armstrong, S H, Jr., Ferry, R M., and Hughes, W. L, Jr : Properties and functions of the purified proteins of animal and human plasmas. In: Blood Substitutes and Blood Transfusion, ed. by S. Mudd and W. W. Thalhimer, Chap XX, p 173 Springfield, Ill, Thomas, 1942.
3. Cohn, E J · The plasma proteins: Their properties and functions. Tr. & Stud, Coll. Physicians, Philadelphia *10*, 149, 1942.
4. Cohn, E. J · Blood, blood derivatives, and blood substitutes. Proc. Am Phil. Soc. *88*, 159, 1944
5 Cohn, E. J : Blood proteins and their therapeutic value. Science *101*, 51, 1945.
6 Cohn, E. J, Luetscher, J A., Jr, Oncley, J. L, Armstrong, S. H, Jr, and Davis, B. D.· Preparation and properties of serum and plasma proteins III. Size and charge of proteins separating upon equilibration across membranes with ethanol–water mixtures of controlled pH, ionic strength and temperature J Am Chem. Soc. *62*, 3396, 1940
7 Cohn, E. J.: The separation of blood into fractions of therapeutic value Ann Int. Med *26*, 341, 1947
8 Cohn, E. J · The history of plasma fractionation. In: Advances in Military Medicine, Vol. I, Chap. XVIII, p. 364. Boston, Little, Brown, 1948
9. Davis, H. A, and Eaton, A. G.: Intravenous administration of bovine serum albumin as a blood substitute in experimental secondary shock Proc. Soc. Exper Biol. & Med. *49*, 20, 1942
10. Keys, A, Taylor, H L, and Savage, G. M.: Utility of animal blood in preparation of plasma for transfusion. J A. M. A [Letter] *117*, 62, 1941
11 Kremen, A J, Hall, H, Kuschnitze, H R, Stevens, B, and Wangensteen, O H. Studies on the intravenous administration of whole bovine plasma and serum to man Surgery *11*, 333, 1942
12 Kremen, A J, Taylor, H L, and Hall, H. Skin sensitivity of man to bovine plasma and its albumin and globulin fractions Proc. Soc. Exper Biol & Med *43*, 532, 1940
13. Savage, G. M., Taylor, H L, and Keys, A Cutaneous reaction to animal plasma proteins in man. J Immunol. *45*, 261, 1942.
14. Taylor, H L, and Keys, A · Bovine albumin as antigen Proc Soc Exper Biol. & Med *50*, 325, 1942
15 Taylor, H L, Keys, A, and Savage, G · Intravenous administration of bovine plasma albumin Proc Soc Exper Biol & Med *50*, 328, 1942
16. Wangensteen, O H, Hall, H, Kremen, A, and Stevens, B. Intravenous administration of bovine and human plasma to man Proof of utilization. Proc. Soc Exper. Biol & Med *43*, 616, 1940.

Human Plasma Fractions

17. Brand, E, Kassell, B., and Saidel, L J.: Chemical, clinical, and immunological studies on the products of human plasma fractionation. III Amino acid composition of plasma proteins. J. Clin Investigation *23*, 437, 1944
18. Brauer, R, and Taylor, H L: Unpublished data
19. Christenson, L R, and MacLeod, C. M: Proteolytic enzyme of serum: Characterization, activation, and reaction with inhibitors J. Gen. Physiol. *23*, 559, 1945
20. Cohn, E J, Hughes, W. L, Jr, and Weare, J. Preparation and properties of serum and plasma proteins XIII. Crystallization of serum albumins from ethanol–water mixtures J. Am Chem Soc *69*, 1753, 1947.
21. Cohn, E J, and Koechlin, B Unpublished data.
22. Cohn, E J, Oncley, J L, Strong, L. E, Hughes, W L, Jr, and Armstrong, S H, Jr: Chemical, clinical, and immunological studies on the products of human plasma fractionation I The characterization of the protein fractions of human plasma. J. Clin. Investigation *23*, 417, 1944
23. Cohn, E J, Strong, L E, Hughes, W. L, Jr, Mulford, D. L, Ashworth, J N, Melin, M, and Taylor, H. L.: Preparation and properties of serum and plasma proteins IV A system for the separation into fractions of the protein and lipoprotein components of biological tissues and fluids J Am Chem Soc *68*, 459, 1946.
24. Cox, H R., Van der Scheer, J, Aiston, S, and Bohnel, E: The purification and concentration of influenza virus by means of alcohol precipitation J. Immunol. *56*, 149, 1947.
25. Dexter, L, and Haynes, F.: Unpublished data
26. Deutsch, H F, Gosting, L J, Alberty, R. A, and Williams, J. W: Biophysical studies of blood plasma proteins III Recovery of γ-globulin from human blood protein mixtures J Biol Chem *164*, 109, 1946.
27. Hisaw, F L Unpublished data
28. Hughes, W L, Jr. Preparation and properties of serum and plasma proteins XIV An albumin fraction isolated from human plasma as a crystalline mercuric salt J Am Chem Soc *69*, 1836, 1947.
29. Janeway, C. A Immunological and clinical studies on purified proteins of human and animal plasma In: Blood Substitutes and Blood Transfusion, ed by S Mudd and W. Thalhimer, Chap. XXI, p 184. Springfield, Ill, Thomas, 1942
30. Kekwick, R. A, Mackay, M E, and Record, B. R: Fractionation of human plasma with ether Nature *157*, 629, 1946
31. Martin, N H, and Derouaux, G: Unpublished data
32. Oncley, J L, Melin, M., Richert, D A., Cameron, J W, and Gross, P. M, Jr: Preparation and properties of serum and plasma proteins. XIX. The separation of the antibodies, isoagglutinins, prothrombin, plasminogen, and X-proteins into subfractions of human plasma. J. Am. Chem. Soc *71*, 541, 1949.

33. Oncley, J. L., Scatchard, G., and Brown, A.: Physical-chemical characteristics of certain of the proteins of normal human plasma J. Phys & Colloid Chem *51*, 184, 1947.
34. Pillemer, L, and Mulford, D : Unpublished data
35: Richert, D A Unpublished data
36 Schade, A J, and Caroline, L An iron-binding component in human blood plasma Science *104*, 340, 1946
36a. Strong, L E.. In Encyclopedia of Chemical Technology, Vol II New York, Interscience, 1948.
37 Taylor, F. H. L.. Unpublished data

Clinical Use—Fibrinogen, Thrombin, and Their Products

38 Bailey, O T, and Ingraham, F D Chemical, clinical, and immunological studies on the products of human plasma fractionation XXI The use of fibrin foam as a hemostatic agent in neurosurgery; clinical and pathological studies J Clin Investigation *23*, 591, 1944
39 Bailey, O T, and Ingraham, F. D. Chemical, clinical, and immunological studies on the products of human plasma fractionation. XXII Fibrin films in neurosurgery, with special reference to their use in the repair of dural defects and in the prevention of meningocerebral adhesions. J Clin. Investigation *23*, 597, 1944
40 Bailey, O T, Ingraham, F. D, Swenson, O, Lowrey, J J, and Bering, E A, Jr : Human fibrin foam with thrombin as a hemostatic agent in general surgery Surgery *18*, 347, 1945
41 Bering, E A, Jr Chemical, clinical, and immunological studies on the products of human plasma fractionation XX The development of fibrin foam as a hemostatic agent for use in conjunction with human thrombin J. Clin Investigation *23*, 586, 1944
42 Cronkite, E P, Lozner, E L, and Deaver, J M Use of thrombin and fibrinogen in skin grafting, preliminary report J A M A *124*, 976 1944
43 Dees, J E : Chemical, clinical, and immunological studies on the products of human plasma fractionation XVIII Fibrinogen coagulum as an aid in the operative removal of renal calculi J Clin Investigation *23*, 576, 1944
44 Edsall, J. T, Ferry, R M, and Armstrong, S H, Jr. Chemical, clinical and immunological studies on the products of human plasma fractionation XV The proteins concerned in the coagulation mechanism J Clin Investigation *23*, 557, 1944
45 Ferry, J D, and Morrison, P R Chemical, clinical, and immunological studies on the products of human plasma fractionation XVI Fibrin clots, fibrin films, and fibrinogen plastics J Clin. Investigation *23*, 566, 1944
46 Ferry, J. D, and Morrison, P R Preparation and properties of serum and plasma proteins VIII The conversion of human fibrinogen to fibrin under various conditions J Am Chem Soc *69*, 388, 1947

47 Ferry, J D, and Morrison, P. R · Preparation and properties of serum and plasma proteins IX. Human fibrin in the form of an elastic film J Am Chem. Soc 69, 400, 1947
48 Ferry, J D, Singer, M, Morrison, P. R, Porsche, J D, and Kutz, R L · Preparation and properties of serum and plasma proteins X. Modification of the physical and chemical properties of fibrin film by heat treatment J. Am Chem Soc 69, 409, 1947
49 Frantz, V. K. Absorbable cotton, paper, and gauze (oxidized cellulose) Ann Surg 118, 116, 1943
50 Hawn, C v. Z, Bering, E A, Jr, Bailey, O T, and Armstrong, S H, Jr Chemical, clinical, and immunological studies on the products of human plasma fractionation XIX A note on the use of fibrinogen and thrombin in the surface treatment of burns J Clin Investigation 23, 580, 1944
51 Hawn, C. v Z, and Porter, K R · The fine structure of clots formed from purified bovine fibrinogen and thrombin. A study with the electron microscope J Exper Med 86, 285, 1947
52 Ingraham, F. D, Alexander, E A, Jr, and Matson, D D · Polyethylene, a new synthetic plastic for use in surgery J A M A 135, 82, 1947
53 Ingraham, F D, and Bailey, O T The use of products prepared from human fibrinogen and human thrombin in neurosurgery Fibrin foams as hemostatic agents, fibrin films in repair of dural defects and the prevention of meningocerebral adhesions J Neurosurg 1, 23, 1944
54 Ingraham, F D, and Bailey, O T Clinical use of products of human plasma fractionation III The use of products of fibrinogen and thrombin in surgery J A M A 126, 680, 1944
55 Ingraham, F D, Bailey, O T, and Cobb, C A, Jr The use of fibrin films as a dural substitute and in the prevention of meningocerebral adhesions Further studies and clinical results J A M A 128, 1088, 1945
56 Ingraham, F D, Bailey, O T, and Nulsen, F E Studies on fibrin foam as a hemostatic agent in neurosurgery, with special reference to its comparison with muscle J Nuerosurg 1, 171, 1944
57 Jenkins, H P, and Clarke, J S. Gelatin sponge, a new hemostatic substance Arch Surg 51, 253, 1945
58 Losch, P K Use of fibrin foam with thrombin in dental operations J Oral Surg 3, 177, 1945
59 Morrison, P R, and Singer, M · Chemical, clinical, and immunological studies on the products of plasma fractionation. XVII A note on the absorption rates of fibrin films in tissues J Clin Investigation 23, 573, 1944
60 Parfentjev, I A A globulin fraction in rabbits' plasma possessing a strong clotting property Am J M Sc 202, 578, 1941
61 Pilcher, C., and Meacham, W F Absorbable gelatin sponge and thrombin for hemostasis in neurosurgery. Surg, Gynec & Obst 61, 365, 1945
62 Putnam, T J The use of thrombin on soluble cellulose in neurosurgery Ann Surg 118, 127, 1943

63 Quinby, W. C, and Landsteiner, E K.: Fibrin foam as hemostatic agent in suprapubic prostatectomy. New England J. Med *233*, 267, 1945.
64. Seegers, W. H.: Purification of prothrombin and thrombin: Chemical properties of purified preparation. J. Biol. Chem *136*, 103, 1940.
65 Singer, M.: Combined use of fibrin film and clot in end to end union of nerves, experimental study. J. Neurosurg. *2*, 102, 1945
66 Swenson, O, and Gross, R.: Absorbable fibrin tubes for vein anastomosis Surgery *22*, 137, 1947.
67. Woodhall, B.. Fibrin foam as hemostatic agent in rehabilitation neurosurgery. J. A. M. A *126*, 469, 1944

Clinical Use—Fraction I in Hemophilia

68. Diamond, L K., and Borges, W.: Unpublished data
69. Lozner, E. L, Kark, R., and Taylor, F. H L.: The coagulation defect in hemophilia: activity in hemophilia of Berkefelded normal human plasma free from fibrinogen and prothrombin. J. Clin Investigation *18*, 603, 939.
70 Lozner, E. L, and Taylor, F H. L: The coagulation defect in hemophilia: Studies on the clot promoting activity associated with plasma euglobulin in hemophilia J. Clin Investigation *18*, 821, 1939
71 Minot, G. R, Davidson, C S, Lewis, J H., Tagnon, H J, and Taylor, F. H L: The coagulation defect in hemophilia. Effect in hemophilia, of parenteral administration of fraction of plasma globulins rich in fibrinogen J. Clin. Investigation *24*, 704, 1945
72 Minot, G R, and Taylor, F. H L Hemophilia: The clinical use of antihemophilic globulin Ann Int Med. *26*, 363, 1947.
73. Pohle, F. J, and Taylor, F. H L.. Coagulation defect in hemophilia Effect in hemophilia of intramuscular administration of globulin substance derived from normal human plasma J Clin. Investigation *16*, 741, 1937
74 Pohle, F. J., and Taylor, F. H L: Coagulation defect in hemophilia Studies on refractory phase following repeated injections of globulin substance derived from normal human plasma in hemophilia J. Clin Investigation *17*, 779, 1938
75 Taylor, F H L, Davidson, C S, Tagnon, H J, Adams, M A, MacDonald, A. H, and Minot, G R Studies in blood coagulation: The coagulation properties of certain globulin fractions of normal human plasma *in vitro* J Clin Investigation *24*, 698, 1945

Clinical Use—Isohemagglutinins and Rh Typing Globulins

76. Cameron, J. W., and Diamond, L K Chemical, clinical, and immunological studies on the products of human plasma fractionation XXIX Serum albumin as a diluent for anti-Rh typing reagent J Clin Investigation *24*, 793, 1945
77. DeGowin, E L. Chemical, clinical, and immunological studies on the products of human plasma fractionation XIV Appraisal of isohemagglutinin activity J Clin Investigation *23*, 554, 1944

78 Diamond, L K, and Denton, R L: Rh agglutination in various media with particular reference to the value of albumin J Lab & Clin. Med *30*, 821, 1945
79. Klendoshoj, N C, and Witebsky, E: Transfusion of O blood conditioned by the addition of blood group specific substances J A M A. *128*, 1091, 1945
80 Melin, M.: Chemical, clinical, and immunological studies on the products of human plasma fractionation XXV. The preparation of anti-A isoagglutinin reagents from mixed blood of groups O and B J. Clin Investigation *24*, 662, 1945
81 Oncley, J L, Melin, M, Cameron, J W, Richert, D A, and Diamond, L K · Methods for the preparation of anti-A, anti-B, and anti-Rh isoagglutinin reagents Ann. New York Acad Sc *46*, 899, 1946
82 Pillemer, L. B · The separation and concentration of isohemagglutinins from human serums Science *97*, 75, 1943
83 Pillemer, L, Oncley, J L, Melin, M, Elliott, J, and Hutchinson, M C · Chemical, clinical and immunological studies on the products of human plasma fractionation XIII The separation and concentration of isohemagglutinins from group-specific human plasma J. Clin Investigation *23*, 550, 1944
84 Thalhimer, W, and Myron, S A.· Globulin fractions of A and B agglutinating serums for blood typing; rapid card technique furnishing permanent record J. A M A *118*, 370, 1942.

Clinical Use—Immune Bodies

85 Adams, J M, and Smith, N. Clinical trial of gamma globulin in the prevention of common respiratory diseases Proc Soc. Exper. Biol & Med. *63*, 446, 1946
86 Anderson, S G, and Ket, W. M The use of gamma globulin in the prophylaxis of measles M J Australia *2*, 196, 1946
87 Aycock, W. L, and Ingalls, T. Maternal disease as a principle in epidemiology of congenital anomalies: With review of rubella Am J M Sc. *212*, 366, 1946
88 Bahlke, A M, and Perkins, J E.: Gamma globulin for preparalytic poliomyelitis J A M A *129*, 1146, 1945
89 Berenberg, W, and Janeway, C A Unpublished data
90. Bradford, W. L, Scherp, H W, and Brooks, A M Prophylactic value of refined antipertussis rabbit serum Proc Soc Exper Biol & Med *49*, 157, 1942
91 Bridgman, W B: Peptic digestion of human gamma globulin. J Am Chem Soc *68*, 859, 1946
92 Deutsch, H F, Petermann, M. L, and Williams, J. W: Biophysical studies of blood plasma proteins II The pepsin digestion and re-
cc [illegible]
93 Dur [illegible]
E [illegible]
agent against homologous serum hepatitis Am J M Sc *213*, 53, 1947.

94 Enders, J. F Chemical, clinical, and immunological studies on the products of human plasma fractionation. X. The concentrations of certain antibodies in globulin fractions derived from human blood plasma J Clin Investigation *23*, 510, 1944.
95. Enders, J F. Mumps Techniques of laboratory diagnosis, tests for susceptibility and experiments on specific prophylaxis. J. Pediat *29*, 129, 1946
96 Felton, H M · Status of passive immunization and treatment in pertussis by use of human hyperimmune serum J A. M A *128*, 26, 1945
97 Gellis, S S, McGuinness, A. C, and Peters, M. A · Study of the prevention of mumps orchitis by gamma globulin Am J M. Sc. *210*, 661, 1945
98 Gellis, S. S, Stokes, J, Jr, Brother, G M, Hall, W. M, Gilmore, H R, Beyer, E, and Morrisey R. A The use of human immune serum globulin (gamma globulin) in infectious (epidemic) hepatitis in the Mediterranean Theater of Operations I Studies of prophylaxis in two epidemics of infectious hepatitis J A M. A *128*, 1062, 1945
99 Gellis, S S, Stokes, J, Jr, Forster, H W., Jr, Brother, G M., and Hall, W M *Use of human immune serum globulin (gamma globulin) in* infectious (epidemic) hepatitis in Mediterranean Theater of Operations II Studies on treatment in epidemic of infectious hepatitis J A M A *128*, 1158, 1945
100 Greenberg, M, Frant, S, and Rutstein, D D "Gamma globulin" and "placental globulin" A comparison of their effectiveness in the prevention and modification of measles J A M A *126*, 944, 1944
101 Grossman, E. B, Stewart, S G, and Stokes, J, Jr Post-transfusion hepatitis in battle casualties and study of its prophylaxis by means of human immune serum globulin J A M A *129*, 991, 1945
102 Havens, W P, Jr, and Paul, J R Prevention of infectious hepatitis with gamma globulin J A M A *129*, 270, 1945
103 High, R H, Anderson, N A, and Nelson, W E Further observations of epidemic diarrhea of newborn, observation of biphasic type of clinical course, therapeutic measures including prophylactic and therapeutic use of gamma globulin J Pediat *28*, 407, 1946
104 Janeway, C A Clinical use of products of human plasma fractionation, albumin in shock and hypoproteinemia, and γ-globulin in measles J A. M A *126*, 674, 1944
105 Janeway, C A Use of concentrated human serum γ-globulin in the prevention and attenuation of measles Bull New York Acad Med *21*, 202, 1945
106 Janeway, C. A, and Poole, V Unpublished data
107. Kramer, S D : Unpublished data
108 Lapin, J H Serum in prophylaxis of contacts and treatment of whooping cough J Pediat *26*, 555, 1945
109 McGuinness, A C, Armstrong, J G, and Felton, H M Hyperimmune whooping cough serum J Pediat *24*, 249, 1944
110 Memorandum prepared by Medical Officers of the Ministry of Health Homologous serum jaundice Lancet *1*. 83. 1943

111. Ordman, C R, Jennings, C G, and Janeway, C A.: Chemical, clinical, and immunological studies on the products of human plasma fractionation XII. The use of concentrated normal human serum gamma globulin (human immune serum globulin) in the prevention and attenuation of measles. J Clin Investigation *23*, 541, 1944
112 Paul, J R · Infectious hepatitis In Nelson Loose-Leaf Medicine, Vol II, Chap 46, p. 213 New York, Nelson
113 Petermann, M L · The splitting of human gamma globulin antibodies by papain and bromelin. J Am. Chem Soc *68*, 106, 1946.
114 Rothbard, S : Bacteriostatic effect of human sera on group A streptococci I Type-specific antibodies in sera of patients convalescing from group A streptococcal pharyngitis J Exper Med *82*, 93, 1945
115 Spurling, N, Shone, J, and Vaughan, J · Homologous serum jaundice. Brit M J *1*, 409, 1946
116 Stillerman, M, and Thalhimer, W Attack rate and incubation period of measles Am J Dis Child *67*, 15, 1944
117 Stokes, J, Jr.· The use of gamma globulin from large pools of adult plasma in certain infectious diseases Ann Int Med *26*, 353, 1947
118 Stokes, J, Jr, and Maris, E P · Personal communication
119 Stokes, J., Jr, Maris, E P., and Gellis, S S Chemical, clinical, and immunological studies on the products of human plasma fractionation XI The use of concentrated normal human serum gamma globulin (human immune serum) globulin in the prophylaxis and treatment of measles J Clin Investigation *23*, 453, 1944
120 Stokes, J, Jr, Maris, E P, and Scott, T McN Personal communication
121 Stokes, J, Jr, O'Neil, G C, Shaffer, M F, Rake, G, and Maris, E. P · Studies on measles IV Results following inoculation of children with egg-passage measles virus J Pediat *22*, 1, 1943
122 Stokes, J, Jr, and Neefe, J R The prevention and attenuation of infectious hepatitis by gamma globulin J A M A *128*, 144, 1945
123 Swan, C, and Tostevin, L Congenital abnormalities following infectious disease in pregnancy, with special reference to rubella M. J. Australia *1*, 645, 1946
124. Sweet, L K, and Hickman, T L Use of normal serum gamma globulin antibodies (human) concentrated (immune serum globulin) in prevention and attenuation of measles J. Pediat *28*, 566, 1946
125 Sweet, L K, Howell, M J, McMurray, L. G, McMain, W A, Jr., and Antell, G J · Use of normal serum gamma globulin antibodies (human) concentrated (immune serum globulin) in treatment of premature infants J Pediat *28*, 571, 1946
126 Yannet, H, and Deutsch, J · Gamma globulin not effective in prophylaxis of epidemic respiratory infections J A M A *131*, 593, 1946.
127 Williams, J. W, Petermann, M. L, Colovos, G C, Goodloe, M B, Oncley, J L, and Armstrong, S H, Jr · Chemical, clinical, and immunological studies on the products of human plasma fractionation II Electrophoretic and ultracentrifugal studies of solution of human

94 Enders, J. F · Chemical, clinical, and immunological studies on the products of human plasma fractionation. X. The concentrations of certain antibodies in globulin fractions derived from human blood plasma J Clin. Investigation *23*, 510, 1944
95. Enders, J F · Mumps: Techniques of laboratory diagnosis, tests for susceptibility and experiments on specific prophylaxis J Pediat. *29*, 129, 1946
96 Felton, H M : Status of passive immunization and treatment in pertussis by use of human hyperimmune serum. J A M. A *128*, 26, 1945
97. Gellis, S. S, McGuinness, A. C, and Peters, M A : Study of the prevention of mumps orchitis by gamma globulin Am J. M. Sc. *210*, 661, 1945
98 Gellis, S S, Stokes, J, Jr, Brother, G. M, Hall, W. M, Gilmore, H. R., Beyer, E, and Morrisey R A.: The use of human immune serum globulin (gamma globulin) in infectious (epidemic) hepatitis in the Mediterranean Theater of Operations I Studies of prophylaxis in two epidemics of infectious hepatitis J. A M A *128*, 1062, 1945
99 Gellis, S S, Stokes, J, Jr, Forster, H W, Jr, Brother, G. M, and Hall, W. M Use of human immune serum globulin (gamma globulin) in infectious (epidemic) hepatitis in Mediterranean Theater of Operations II Studies on treatment in epidemic of infectious hepatitis J A. M. A *128*, 1158, 1945
100 Greenberg, M, Frant, S, and Rutstein, D D "Gamma globulin" and "placental globulin" A comparison of their effectiveness in the prevention and modification of measles J A M A *126*, 944, 1944
101 Grossman, E B, Stewart, S G, and Stokes, J, Jr Post-transfusion hepatitis in battle casualties and study of its prophylaxis by means of human immune serum globulin J A M A *129*, 991, 1945
102 Havens, W P, Jr, and Paul, J R Prevention of infectious hepatitis *with gamma globulin J A M A 129, 270, 1945*
103 High, R H, Anderson, N A, and Nelson, W E. Further observations of epidemic diarrhea of newborn; observation of biphasic type of clinical course, therapeutic measures including prophylactic and therapeutic use of gamma globulin J Pediat *28*, 407, 1946
104 Janeway, C A Clinical use of products of human plasma fractionation, albumin in shock and hypoproteinemia, and γ-globulin in measles J A M A *126*, 674, 1944
105 Janeway, C. A · Use of concentrated human serum γ-globulin in the prevention and attenuation of measles Bull New York Acad Med. *21, 202, 1945*
106 Janeway, C A, and Poole, V Unpublished data
107. Kramer, S D Unpublished data
108 Lapin, J. H Serum in prophylaxis of contacts and treatment of whooping cough. J Pediat *26*, 555, 1945
109 McGuinness, A C, Armstrong, J. G, and Felton, H M · Hyperimmune whooping cough serum J Pediat *24*, 249 1944
110 Memorandum prepared by Medical Officers of the Ministry of Health Homologous serum jaundice Lancet *1* 83, 1943

144 Eckhardt, R. D, Lewis, J. H, Murphy, T L, Batchelor, W. H, and Davidson, C S.. Chemical, clinical, and immunological studies on the products of human plasma fractionation XXXIV. Comparative studies on nutritive value of orally and intravenously administered human serum albumin in man. J. Clin Investigation *27*, 119, 1948
145. Elman, R : Personal communication.
146. Ferrebee, J W, and Geiman, Q M.. Studies on malarial parasites III A procedure for preparing concentrates of *Plasmodium vivax* J Infect Dis. *78*, 173, 1946
147. Fine, J., Frank, H. A, and Seligman, A · Traumatic shock; studies in therapy and hemodynamics of tourniquet shock J. Clin. Investigation *23*, 731, 1944.
148 Fine, J, Frank, H. A, and Seligman, A · Traumatic shock incurable by volume replacement therapy. Ann. Surg *122*, 652, 1945.
149 Gellis, S. S, Neefe, J. R, Stokes, J, Jr, Strong, L. E, Janeway, C. A, and Scatchard, G : Chemical, clinical and immunological studies on the products of human plasma fractionation. XXXVI Inactivation of the virus of homologous serum hepatitis in solutions of normal human serum albumin by means of heat J Clin Investigation *27*, 239, 1948
150 Gibson, S T Unpublished data
151 Govaerts, P, and Gregoire, P E.: Acta Biol Belg *1*, 530, 1942.
152 Govaerts, P, and Lequime, J · Considérations sur la pathologie des oedemes de famine Bull de l'académie roy méd de Belgique 7, 260, 1942
153. Hawn, C. v. Z, and Janeway, C. A . Histological and serological sequences in experimental hypersensitivity J Exper. Med *85*, 571, 1947.
154. Hegsted, D M., Hay, A L, and Stare, F. J · Chemical, clinical, and immunological studies on the products of human plasma fractionation XXIV. Studies on the nutritive value of human plasma fractions J. Clin Investigation *24*, 657, 1945
155 Heyl, J. T, Gibson, J. G, 2nd, and Janeway, C A Studies on the plasma proteins. V The effect of concentrated solutions of human and bovine serum albumin on blood volume after acute blood loss in man J Clin Investigation *22*, 763, 1943
156 Heyl, J T, and Janeway, C. A . The use of human albumin in military medicine The theoretical and experimental basis for its use U S Nav M Bull. *40*, 785, 1942.
157 Heyl, J. T, and Janeway, C A In preparation
158 Janeway, C A, Moll, G H, Armstrong, S H Jr, Wallace, W. M, Hallman, N, and Barness, L. A Diuresis in children with nephrosis Comparison of response to injection of normal human serum albumin and to infection, particularly measles Trans Ass Am Phys *61*, 108, 1948
159 Janeway, C A Other uses of plasma fractions with particular reference to serum albumin Ann Int Med *26*, 368, 1947
160 Janeway, C A, Gibson S T, Woodruff L M, Heyl J T, ...
and Ne
on the
human

serum albumin and immune serum globulins J Clin Investigation *23*, 433, 944.

Clinical Use—Human Serum Albumin

128. Albright, F, Forbes, A, and Reifenstein, E C, Jr.: The fate of plasma protein injected intravenously. Tr A Am. Physicians *59*, 221, 1946
129. Armstrong, S. H, Jr.: Albumin in internal medicine Analysis of mechanisms of action in terms of effective serum osmotic pressure and of vascular permeability to albumin. Am. J. Med *4*, 390, 1948.
130 Armstrong, S H, Jr, Emerson, K, and Gibson, S. T.: Personal communication.
131. Aub, J C, Zamecnik, P. C, and Nathanson, I T.: Physiologic action of *Clostridium oedematiens* (Novyi) toxin in dogs. J. Clin Investigation *26*, 401, 1947.
132. Ballou, G A, Boyer, P. D., Luck, J. M., and Lum, F. G.: Chemical, clinical, and immunological studies on the products of human plasma fractionation V. The influence of non-polar anions on the thermal stability of serum albumin J. Clin Investigation *23*, 454, 1944.
133 Ballou, G. A, Boyer, P. D, Luck, J M, and Lum, F. G . The heat coagulation of human serum albumin J Biol Chem. *153*, 589, 1944.
134 Ballou, G A, Boyer, P D, and Luck, J. M · Electrophoretic mobility of human serum albumin as affected by lower fatty acid salts J Biol Chem *159*, 111, 1945
135. Beecher, H. K.: *Preparation of battle casualties for surgery Ann Surg* *121*, 769, 1945
136. Boyer, P D, Lum, F G, Ballou, G A, Luck, J M. ,and Rice, R. G : The combination of fatty acids and related compounds with serum albumin. I. Stabilization against heat denaturation. J. Biol. Chem *162*, 181, 1946
137. Boyer, P. D, Ballou, G A, and Luck, J M The combination of fatty acids and related compounds with serum albumin II Stabilization against urea and guanidine denaturation J Biol Chem *162*, 199, 1946
138 Cournand, A, Noble, R P, Breed, E S, Lauson, H D, Baldwin, E D, Pinchot, G B, and Richards, D W, Jr Chemical, clinical, and immunological studies on the products of human plasma fractionation VIII Clinical use of concentrated human serum albumin in shock and comparison with whole blood and with rapid saline infusion J Clin Investigation *23*, 491, 1944
139 DeSanctis, A, and Sullivan, A M . Nephrosis A case treated with concentrated, low salt, human serum albumin J. Pediat *30*, 91, 1947
140. Dubos, R J.: The effect of lipids and serum albumin on bacterial growth J Exper. Med. *85*, 9, 1947
141. Dubos, R J, and Davis, B D Factors affecting growth of tubercle bacilli in liquid media J Exper Med *83*, 409, 1946
142 Dunphy, J E., and Gibson, J G, 2nd. The effect of infusions of bovine serum albumin in experimental shock Surgery *14*, 509, 1943
143 Ebert, R. V., Stead, E A, Jr, and Gibson, J G, 2nd Response of normal subjects to acute blood loss Arch Int Med *68*, 578, 1941

177. Scatchard, G., Batchelder, A. C., and Brown, A.: Chemical, clinical, and immunological studies on the products of human plasma fractionation VI. The osmotic pressure of plasma and of serum albumin. J. Clin. Investigation *23*, 458, 1944.

178. Seligman, A., Frank, H. A., and Fine, J. Hemodynamic effects of alterations of blood viscosity in normal dogs and dogs in shock. J. Clin. Investigation *25*, 1, 1946.

179. Sharp, D. G., Taylor, A. R., McLean, I. W., Jr., Beard, D., and Beard, J. W.: Density and size of influenza virus A (PR8 strain) in solution Science *100*, 151, 1944

180. State, D., Romero, F. T., Castellanos, M. M., and Wangensteen, O. H.: The clinical evaluation of bovine albumin as a blood substitute. Surgery *22*, 424, 1947

181. Stead, E. A., Jr., and Ebert, R. V.: Studies on human abumin. In: Blood Substitutes and Blood Transfusion, ed. by S. Mudd and W. Thalhimer, p. 185 Springfield, Ill., Thomas, 1942

182. Stead, E. A., Jr., Brannon, E. S., Merrill, A. J., and Warren, J. V.: Concentrated human serum albumin in the treatment of shock. Arch. Int. Med. *77*, 564, 1946

183. Taylor, F. H. L., and Davidson, C. S. Personal communication

184. Thorn, G. W., Armstrong, S. H., Jr. and Davenport, V. D.. The use of salt poor concentrated albumin solution in the treatment of hepatic cirrhosis. J. Clin. Investigation *25*, 304, 1946

185. Thorn, G. W., Armstrong, S. H., Jr., Dickerson, V. C., Woodruff, L. M., and Tyler, F. H. Chemical, clinical, immunological studies on the products of human plasma fractionation XXX The use of salt poor concentrated human serum albumin solution in the treatment of chronic Bright's disease J. Clin. Investigation *24*, 802, 1945

186. Vallee, B. L., Hughes, W. L., Jr., and Gibson, J. G., 2nd. A method for the separation of leukocytes from whole blood by flotation on serum albumin Blood *1*, 82, 1947

187. Warren, J. V., Stead, E. A., Jr., Merrill, A. J., and Brannon, E. S. Chemical, clinical, immunological studies on the products of human plasma fractionation IX The treatment of shock with concentrated human serum albumin J. Clin. Investigation *23*, 506, 1944

188. Waterhouse, C., and Holler, J. Metabolic studies on protein depleted patients receiving a large part of their nitrogen intake from human serum albumin administered intravenously J. Clin. Investigation *27*, 560, 1948

189. Williams, R. R.: The relationship of sulfhydryl and disulfide groups to the antigenic power of bovine serum albumin J. Immunol *55*, 161, 1947

190. Woodruff, L. M., and Gibson, S. T. The use of human albumin in military medicine, the clinical evaluation of human albumin U. S. Nav. M. Bull *40*, 791, 1942.

191. Zamecnik, P. C., Nathanson, I. T., and Aub, J. C. Physiologic action of *Clostridium welchii* (Type A) toxins in dogs J. Clin. Investigation *26*, 394, 1947

161. Janeway, C A., and Poole, V. S · Unpublished data
162 Kendrick, D. B, Jr, Reichel, J., Jr., and McGraw, J. J., Jr.: Human serum albumin (concentrated). Clinical indications and dosage Army M. Bull. *68*, 107, 1943
163 Klotz, I. M, Longfellow, J. M, and Johnson, O H: Interactions between proteins and azosulfonamides Science *104*, 264, 1946
164. Krayer, O Unpublished data
165 Kunkel, H G, Labby, D H, Ahrens, E H, Shank, R. E, and Hoagland, C L · The use of concentrated human serum albumin in the treatment of cirrhosis of liver J. Clin Investigation *27*, 305, 1948
166 Lowell, A, Cournand, A, and Richards, D W, Jr.: Changes in plasma volume and mean arterial pressure after the intravenous injection of concentrated human serum albumin in thirty-eight patients with oligemia and hypotension. Surgery *22*, 442, 1947
167. Luetscher, J A, Jr · The effect of a single injection of concentrated human serum albumin on circulating proteins and proteinuria in nephrosis J Clin Investigation *23*, 365, 1944
168 Martin, D S, Erickson, J O, Putnam, F W., and Neurath, H · Native and regenerated bovine albumin II Immunological properties J. Gen. Physiol *26*, 533, 1946
169 McCarthy, M D, and Parkins, W M Comparative effectiveness of albumin, globin, hemoglobin, gelatin, oxypolygelatin, saline, Ringer's, blood and plasma upon the survival of rats subjected to standardized scald burns Am J. Physiol *150*, 428, 1947
170 Moore, F D Personal communication
171. Newhouser, L R, and Lozner, E. L. The use of human albumin in military medicine, the standard Army-Navy package of serum albumin human (concentrated) U S Nav. M. Bull *40*, 796, 1942
172 Patek, A J, Jr, Mankin, H, Colcher, H, Lowell, A, and Earle, D P, Jr Effects of intravenous injection of concentrated human serum albumin on blood plasma, ascites, and renal functions in 3 patients with cirrhosis of the liver J Clin Investigation *27*, 135, 1948
173 Putnam, F W, Erickson, J O, Volhin, E, and Neurath, H. Native and regenerated bovine albumin I Preparation and physicochemical properties J. Gen Physiol *26*, 513, 1943
174 Scatchard, G, Batchelder, A C, and Brown, A Preparation and properties of serum and plasma proteins VI. Osmotic equilibrium in solutions of serum albumin and sodium chloride J Am Chem. Soc *68*, 2320, 1946
175 Scatchard, G, Gibson, S. T, Woodruff, L M, Batchelder, A C, and Brown, A Chemical, clinical, and immunological studies on the products of human plasma fractionation IV. A study of the thermal stability of human serum albumin J Clin Investigation *23*, 445, 1944
176. Scatchard, G., Strong, L E. Hughes, W L, Jr, Ashworth, J N, and Sparrow, A H Chemical, clinical, and immunological studies on the products of human plasma fractionation XXVI The properties of solutions of human serum albumin of low salt content J Clin Investigation *24*, 671, 1945

The Mechanism of Acclimatization to Heat

JEROME W. CONN *
University of Michigan Medical School, and University Hospital, Ann Arbor, Michigan

For at least 175 years (5,16,18) certain adverse effects of heat upon man have been recognized. It has been known, too, that persons native to a tropical or desert environment are much better able to ward off such untoward effects than are new arrivals from cooler climates (16,18) With the development in temperate climates of industries with high environmental temperatures, many of the workers began to suffer the ill effects of heat. These incapacitating effects manifested themselves as the now well-recognized clinical syndromes of heat exhaustion and heat cramp. Among the many facts which large-scale industrial studies (6,7,15,26,27) disclosed were the following:

(*1*) Workers new to hot jobs are much more susceptible to the ill effects of heat than are men who have become accustomed to the job. (*2*) Even in seasoned workers, certain factors appear to lower tolerance for heat, for example, a layoff, vacation, or an alcoholic spree (*3*) Some individuals, despite long-continued exposure, are particularly susceptible, as indicated by repeated episodes of heat sickness (*4*) Most workers gradually acquire an increased capacity to work in heat.

Acclimatization to heat is the phrase now used to denote that process of adaptation which occurs when man is exposed to an environment hotter than that to which he is accustomed and which,

* Work from the author's laboratory referred to in this chapter represents results of the combined efforts of Dr. M W. Johnston, Dr. L. H. Louis, Miss B Steele, Miss B. Johnson, Mr. H. Dirks, Jr, Mrs. S. Wark, Mrs B. Sprunger, and the writer, and was performed under contracts with (*1*) Office of Scientific Research and Development, National Research Council, Washington, D C., (*2*) Medical Research and Development Board, Office of the Surgeon General, United States Army, Washington, D C., and (*3*) Research Grants and Fellowships Division, U. S Public Health Service, Washington, D. C.

This chapter was originally prepared in 1945 and revised in 1947. Time has not permitted an extensive revision in proof, nor is it necessary. Although much work is now in progress which will ultimately fill many of the gaps in this review, several more years will be required before the physiologic role in health and disease of even so well characterized a molecule as serum albumin can possibly be thoroughly studied. Thus, far more information to guide the clinician in an intelligent use of blood derivatives can be expected to appear in the literature in the next few years. Moreover, our understanding of physiologic processes should advance slowly and steadily as the proteins responsible for them are isolated from blood and tissues, characterized, and made available for experimental use. Three recently published reviews not listed in the original bibliography will supplement this chapter to some extent.

192 Cohn, E J · Chemical, physiological, and immunological properties and clinical uses of blood derivatives Experientia *3*, 125, 1947.
193 Janeway, C A.. Clinical use of blood derivatives. J. A. M. A. *138*, 859, 1948
194 Gibson, S. T · Blood and its derivatives New England J. Med. *239*, 544, 579, 1948
195 Seegers, W. H, and Ware, A. G · Purification of prothrombin and thrombin Fed Proc 7, 186, 1948.
196 Foreign letters in J A M A *139*, 731, 803, 1949

such as flushing of the face, neck, and chest, injection of the scleras, edema of nasal mucous membranes, and often edema of the hands and feet. Bazett *et al.* (3,25) have shown that men working effectively in hot environments maintain an increased blood and interstitial fluid volume, and often show an increase in resting cardiac output and an increased peripheral circulation. Taylor *et al* (29) have concluded that the ability of the acclimatized man to work at a lower level of internal temperature is the result of an improved peripheral circulation, and that the cardiovascular adjustment which occurs during the first 4 days of exposure to work in the heat consists of a "vasomotor adaptation" to the new circumstances While the observations and conclusions on the increased efficiency of the cardiovascular system of the acclimatized man are valid, they deal with the results of acclimatization rather than with the mechanisms responsible for its accomplishment.

Similar observations have been made with regard to sweat gland functions It is known, for example, that the fully acclimatized man produces a somewhat larger volume of sweat during the performance of a standard work load in the heat than does the unacclimatized individual (14,22,28) Under conditions of dry heat this constitutes an important aid in eliminating heat from the body by the vaporization of water from the skin In moist heat, however, this advantage is lost, since over 60 per cent of the sweat may drip off the body and thus be of no value in ridding the body of heat. In either case, however, a large sweat volume carries with it a relatively large amount of sodium chloride to the surface of the body So far as is known, sodium chloride in the sweat serves no useful purpose and therefore constitutes a paradox to the rule of physiologic economy Even here, however, an adaptive phenomenon occurs Dill and associates (13) found that as acclimatization to heat progresses the concentration of sodium chloride in sweat diminishes Intrigued by this interesting change in the composition of sweat, we (8–10) have confirmed the results of the Boston group, and have made extensive studies of the phenomenon in an effort to discover the physiologic mechanism responsible for it. It was considered likely that the cardiovascular adjustments associated with the process of acclimatization to heat, involving as they do an increase in total extracellular fluid volume, were related to the large changes which were occurring simultaneously in the electrolyte content of the sweat

when completed, results in a remarkable increase in his capacity to live and work in the heat without distressing symptoms This definition must be qualified with respect to the intensity and duration of the heat load to which the individual is exposed. There is, of course, an upper limit of tolerability to heat for the fully acclimatized man (14,23). As the load on his heat-dissipating mechanisms is increased, a time comes when the limit of his capacity to make certain physiologic adjustments is reached. Beyond this he can no longer successfully cope with his environment. It is within these physiologic limits that the process of acclimatization to heat can be observed

Under conditions of daily work in the heat the process of adaptation is essentially complete within 10 to 14 days, although a small increment of improved performance is detectable in the following 10 to 20 days. The results of full acclimatization to heat are most dramatic. Whereas on the first day of exposure to heat a subject attempting to complete a given work load collapses with high rectal temperature and evidence of peripheral vascular failure, the same individual by the fourth or fifth day of exposure performs the task easily, without any vascular instability and with a much lower rectal temperature. Simple exposure to a hot environment without work confers a lesser degree of acclimatization The degree of acclimatization acquired is related, within limits, to the stress imposed upon heat-dissipating mechanisms.

Careful and painstaking studies have been made by Dill *et al.* (11–13,20,21), Bazett *et al* (3,4,25), Adolph (1), Keys *et al* (19, 28,29), Robinson *et al.* (22,23), and others (2,14,31), in an effort to explain on a physiologic basis the better performance observable in the acclimatized man The major differences detected have involved changes in the functional capacity of the cardiovascular system and in the function of the sweat glands. An unacclimatized man forced to work in the heat imposes a great burden upon his cardiovascular system To serve the purposes of heat elimination, there occurs reflexly a large increase in the minute volume of blood circulating through the skin (1,17) This acute diversion of blood to the periphery produces the early signs of peripheral vascular collapse, namely, rapid pulse rate (12,17), decreased stroke volume and sometimes decreased minute output (2), severe postural hypotension (14,17,31) and peripheral evidence of vascular engorgement,

of 5 Gm. of salt. On this same day, no change occurred in the concentration of salt in the sweat, so that a negative salt balance resulted. Within the next 24 to 36 hours, however, a sharp decrease occurs in the concentration of salt in sweat This saves the day!

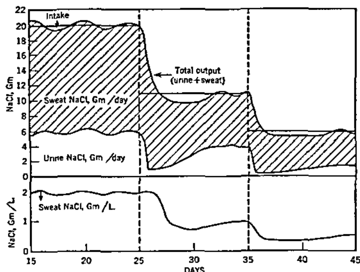


Fig 1 Effects of sharp decreases of sodium chloride intake upon sodium chloride metabolism of men acclimatized to work in the heat Sweat volume, 7 liters per day

At the new level of salt in the sweat and the continuing low level of urinary salt, a short period of positive salt balance ensues. At least some of the salt lost in the negative balance phase is replaced. Then follows a gradual rise of urinary output of salt until the losses of salt in urine and sweat approximate the intake. Salt balance is now restored, and the newly acquired lower level of salt in the sweat continues. The same phenomenon occurs again when the salt intake is cut sharply from 11 to 6 Gm. per day. The physiologic lag manifested by the sweat glands in reducing the salt content of sweat correlates well with the time required to effect such a decrease by the use of desoxycorticosterone acetate (see below).

Under the conditions with which we worked with acclimatized

Conservation of Sodium Chloride by Fully Acclimatized Men

Man unacclimatized to heat and living in a temperate climate produces sweat which contains, on the average, about 4 Gm. of sodium chloride per liter. There is considerable variation among individuals, in addition to which the season of the year, the degree of activity, and the sodium chloride intake are important influences. With diet constant and containing 15 Gm. of sodium chloride, full acclimatization to work in the heat results in a decrease of 60 to 70 per cent in the concentration of sodium chloride in sweat. Thus, a man producing 8 L. of sweat per day would diminish his skin loss of salt from 32 Gm to 9 5 to 13 Gm. This new state of affairs allows the subject to maintain salt equilibrium on the diet that he is being fed. He has made an adjustment related, as will be seen below, to two factors: his salt supply and his total daily sweat production

In an effort to discover the limit of this adaptive mechanism, we performed experiments upon fully acclimatized men whereby the total daily production of sweat remained reasonably constant but *in which periodic decreases in the salt intake were made. Other* constituents of the diet remained constant. Figure 1 is a schematic representation of the phenomena which were observed. For the sake of simplicity, only three levels of salt intake are shown in the illustration, namely, 20, 11, and 6 Gm. per day. Many other levels, ranging from 20 to 1 9 Gm. per day, brought the same physiologic adjustments. It will be noted that at a daily intake level of 20 Gm. of sodium chloride its concentration in the sweat approximated 2 Gm. per liter. About 14 Gm of salt were lost in the sweat and the remaining 6 Gm. appeared in the urine. Salt balance was maintained

An abrupt decrease of the salt intake from 20 to 11 Gm. per day results in an interesting chain of events. On the first day, there is a very sharp fall in the concentrations of sodium and chloride in the urine, the total amounting to less than 1 Gm. Since it is unlikely that this active renal tubular reabsorption of salt could have occurred in the absence of well-functioning adrenal cortices, the phenomenon constituted an initial clue to the mechanism of the physiologic adjustment. At any rate, this renal mechanism for the conservation of body salt was able on the first day to effect a saving

and night) in a simulated tropical environment. Hard work was performed during the day, beginning on the first day with light work and arriving at hard work by the fourth day. The horizontal

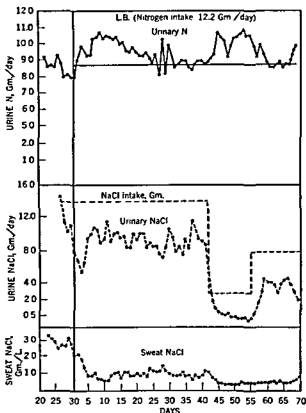


Fig 2 Phenomenon of negative nitrogen balance during acclimatization to heat and its reappearance, after acclimatization, upon sharp restriction of sodium chloride intake.

line represents the amount of nitrogen which might be expected in the urine, were the subject in nitrogen equilibrium The formula for it is

N intake − (stool N + sweat N) = expected urine N for balance

The phase of negative nitrogen balance extended over a period of 25 days, after which the subject came into nitrogen equilibrium.

men, the limit of the sweat glands' ability to reabsorb salt produced sweat values of 0.25 to 0.35 Gm. of sodium chloride per liter. This occurred with diets containing 1.9 to 3.2 Gm. of salt per day in men sweating 5 to 9 L. per day. Under these circumstances, the urine contained as little as 50 mg. of salt per day, and the ability to maintain salt balance varied from man to man. Under other conditions, however, we have been able to obtain sweat as dilute in sodium chloride as 0.10 Gm. per liter.

The chain of adjustments described above can be seen to work in reverse when the proper conditions are applied. Under the circumstances of low salt intake, low sweat salt concentration, low urinary salt excretion, and continued existence in the heat, when work periods are discontinued for several days thus abruptly lowering total sweat volume, there occurs *(1)* a rapid rise in urinary salt, followed by *(2)* an increase in the concentration of salt in the sweat.

Thus, an adaptive mechanism is available to man working in heat whereby he can reduce salt losses from his body, when conditions are sufficiently severe, to as little as 5 per cent of the original loss. A major portion of this saving accompanies the process of acclimatization. But further adaptability, with respect to salt conservation, can be demonstrated in men usually considered to be fully acclimatized to work in heat. The metabolic changes which accompany "superacclimatization" are the same as those observed in the process of acclimatization.

Nitrogen and Salt Equilibriums during Acclimatization to Heat

Early in this work we had found that the process of acclimatization to heat was invariably accompanied by negative nitrogen balance (10). This loss of nitrogen may amount to as much as 7 Gm. per day, but usually it is in the range of 1.5 to 3 Gm. per day. The period of negative nitrogen balance begins with the initial day of work in the heat, continues for 2 to 4 weeks, and is independent of the nitrogen intake, occurring at high as well as at medium levels of protein feeding.

Figure 2 illustrates this phenomenon. The upper part of the chart deals with nitrogen studies. The vertical line represents the time that the subject began a 70 day period of continued residence (day

normal individuals is known to produce a sharp decrease in the concentration of urinary sodium and chloride. This effect, however, is observed only during the first 2 or 3 days of injections, and is followed by a rebound of the urinary sodium and chloride values to much higher levels, despite continued administration of the steroid. It has also been established that desoxycorticosterone has little or no effect upon protein metabolism.

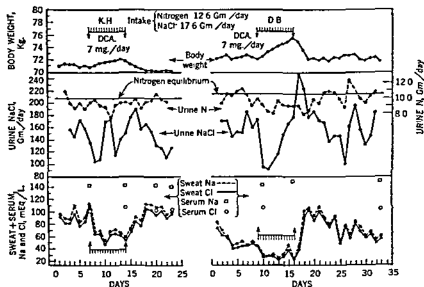


Fig 3 Sodium chloride acclimatization induced with desoxycorticosterone acetate in men living in a temperate climate Note the absence of negative nitrogen balance.

It seemed important, therefore, to determine the effects of desoxycorticosterone in unacclimatized men living in a temperate climate. For if during acclimatization to heat the mechanism of the decreased salt concentration in sweat is an increased adrenocortical activity, exogenous desoxycorticosterone might be expected to effect a similar lowering of such salt concentration in the absence of exposure to heat.

Figure 3 demonstrates the metabolic effects of such experiments upon 2 unacclimatized men living in a temperate climate. The lower portion of the chart shows the intense effect of DCA in lowering the sodium and chloride contents of sweat. The effect becomes notice-

Examination of what was occurring simultaneously with respect to sodium chloride in sweat and urine reveals that the chain of events schematically illustrated previously in Figure 1 was taking place, namely, a sharp initial fall in the urinary excretion of salt, followed by a drop in the concentration of salt in the sweat. The total loss of salt via the sweat having now been greatly reduced, urinary excretion of salt climbed again to the equilibrium level.

Between the twenty-fifth and the forty-first day, nitrogen and sodium chloride equilibrium was maintained. The subject continued to perform his work efficiently and could be said by any standards to have become fully acclimatized to work in the heat.

It was decided then to load the adaptive mechanism further by sharply reducing the total intake of salt from 14.5 to 2.9 Gm. per day. All other elements of the diet remained constant, as did the daily work It can be seen from the graph that the entire acclimatization pattern of metabolism returned, namely, a negative nitrogen balance in association with sharply falling levels of sodium chloride in urine and sweat. In this 13 day period, the salt conserving mechanisms were pushed practically to their limits. Urine salt gradually fell to below 500 mg per day and salt concentration in the sweat remained around 300 mg. per liter. On the fifty-fifth day, when salt was added to the diet, there occurred a prompt rise in urinary salt The acute call for salt conservation was relieved. Over the next 5 days, the negative nitrogen balance gradually disappeared The newly acquired low level of salt in the sweat was maintained for many days before it began to rise slowly.

This interesting metabolic pattern (negative nitrogen balance and sharply falling concentrations of sodium chloride in sweat and urine) in response to the need to conserve salt, was highly suggestive of a sudden increase in adrenocortical activity. If this proved to be the case, deductive reasoning would lead to another tentative conclusion. Since electrolyte and protein metabolism are simultaneously affected, indicating that cortical steroids of both the desoxy and the "11 oxy" types are active, the initial stimulus for activation of the adrenal cortices is probably mediated via pituitary adrenocorticotropic stimulation.

Metabolic Effects of Desoxycorticosterone Acetate

On Unacclimatized Men Living in a Temperate Climate. Daily administration of desoxycorticosterone acetate (DCA) to

tropic activity and if an endogenous desoxylike steroid is responsible for the fall of sweat and urinary salt concentrations during the process of acclimatization to heat, the metabolic pattern obtained in the desoxycorticosterone experiments described above are clearly explainable.

On Men in the Process of Acclimatization to Heat. It was believed that DCA could be used as a tool in laying bare the

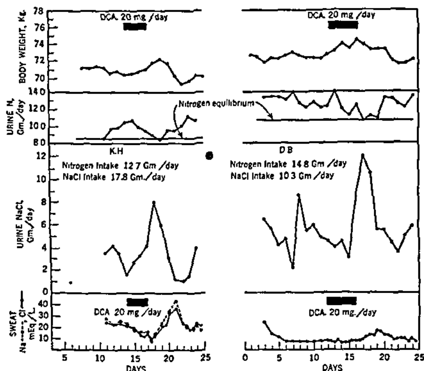


Fig 4 Effect of desoxycorticosterone acetate administration on the metabolic pattern characteristic of the acquisition of acclimatization

mechanism of acclimatization to heat because of its unique characteristics of affecting electrolyte metabolism intensely without exerting a significant effect upon protein metabolism.

Figure 4 shows the effects of DCA given to 2 men who were already in the process of acclimatizing to heat and were exhibiting

able 18 to 36 hours after injection of the initial dose. What appears to us significant is that when the effect of the last injection has worn off (in 2 to 4 days) there occurs an abrupt rise in the salt content of the sweat to a level frequently exceeding the highest levels obtained before the administration of DCA. This suggests that administration of the exogenous hormone temporarily depresses the normal activity of the adrenal cortices. Such depression would be expected to be mediated by way of decreased pituitary activity in producing adrenocorticotropic substance. And, indeed, the Sayers (24) have indicated that in rats administration of DCA decreases adrenocorticotropic activity. In this connection, it may be significant that a positive nitrogen balance, although small in degree, was evident during the period of DCA administration (Fig. 3). This observation supports the thesis of DCA depression of adrenocorticotropic activity. The curves for urinary sodium chloride show clearly the sharp fall and the rebound of these levels, despite continuation of DCA.

Two more points are worthy of mention with regard to the data obtained in this experiment. First, although the kidney "breaks through" the salt-lowering effect of DCA on the urine, the sweat glands continue to secrete a fluid which is dilute in sodium and chloride as long as the DCA is administered Second, the sharp fall in the concentration of sodium and chloride in sweat, produced under the influence of DCA, occurs in the absence of any change in the blood levels of these constituents. This means that the sweat glands are performing active osmotic work in the reabsorption of these elements, and that this process has been stimulated by the pharmacologic activity of DCA.

It can be concluded, then, that exogenous desoxycorticosterone produces the same effects upon the sodium and chloride contents of sweat and urine as those which are observed to occur spontaneously when man is becoming acclimatized to heat There is, however, a significant difference in the over-all metabolic pattern, particularly with reference to nitrogen metabolism. Whereas man acclimatizing [illegible] for nitrogen, subjects [illegible] climate by the use of [illegible] e contrary, they tend toward a retention of nitrogen If DCA depresses adrenocortico-

activity in response to work in the heat, though not sharply defined, is clearly related to the need for conserving body salt. In this particular instance, the protein catabolic effect appears to be merely an accompanying manifestation of increased adrenocortical activity. When the need to produce large amounts of salt-retaining cortical steroids is diminished by the administration of exogenous DCA or sufficient salt, the protein catabolic effect soon disappears.

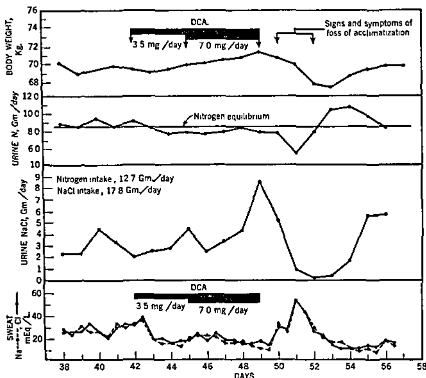


Fig 5 Temporary destruction of full acclimatization by the administration of desoxycorticosterone acetate

(5) The physiologic process of acclimatization to heat is impeded by substituting exogenous adrenocortical steroids for endogenous ones While the peripheral effects of the administered steroid are similar to those observed in full acclimatization, cessation of such substitution discloses depression of the pituitary–adrenal mecha-

the characteristic metabolic pattern of progressing acclimatization at the time that DCA administration was begun. The effect of DCA upon the salt content of urine and sweat was similar to that observed in men who were not acclimatizing to heat. But the negative nitrogen balance characteristic of the process of acclimatization was influenced by the DCA, the degree of negative balance being considerably lessened Since DCA, as such, has no effect upon nitrogen metabolism, this effect must be regarded as an indirect one Depression by DCA of *pituitary adrenocorticotropic activity would bring* this about by decreasing the release of endogenous cortical steroids, both of the salt-retaining variety and of those which exert a protein catabolic effect The decreased release of the endogenously produced salt-retaining steroids is temporarily masked, however, by the peripheral activity of the DCA administered. But it is not masked for long! As the effect of the exogenous steroid wanes (within 2 to 3 days after the last injection—see Fig 4), the concentration of salt in the sweat bounds upward and often exceeds the level observed before acclimatization was begun After several more days, the salt content of sweat begins to fall again This second fall is accompanied by a return of a negative nitrogen balance The subject is now beginning to acclimatize all over again, the process of acclimatization having been interrupted and interfered with by a short period of DCA administration

From these and similar observations it seems reasonable to conclude:

(*1*) The mechanism by which man adapts himself to physical work in the heat consists of increased physiologic activity of the adrenal cortices in response to enhanced release or activity of the pituitary adrenocorticotropic hormone.

(*2*) Increased adrenocortical activity is evidenced by a metabolic pattern consisting of the simultaneous appearance of salt-retaining influences and a protein catabolic effect

(*3*) Since both the salt-retaining and the protein catabolic effects can be reversed by administration of desoxycorticosterone acetate, which, in itself, has no effect upon protein metabolism, the depressing effect of DCA is exerted upon the production of pituitary adrenocorticotropic principle rather than upon adrenal function directly

(*4*) *The stimulus which initiates increased adrenocorticotropic*

the fifty-fourth day, performance was normal; so, too, was the mechanism for salt conservation. As usual, a slight lag occurred in the return to nitrogen equilibrium.

In attempting to interpret these metabolic changes in terms of endocrine function and in the light of the thesis developed on the basis of the previous experiment speculation about one point is unavoidable: What is the mechanism by which nitrogen equilibrium is finally established in the fully acclimatized man? The metabolic and physical responses observed following cessation of a period of DCA administration made it obvious that full acclimatization was temporarily interrupted by the procedure. DCA inhibition of the pituitary–adrenal interrelationship explains the phenomenon adequately. This implies that in the fully acclimatized man working and living in the heat, there exists a continuously higher level of activity of the pituitary-adrenal mechanism than is present in man living in a temperate climate. The persistently low level of sodium and chloride in sweat is one indication of this Why, then, does the phase of negative nitrogen balance, characteristic of the acclimatizing man, disappear in 3 to 4 weeks and return to the equilibrium level?

The phenomenon has been observed in other connections, but a precise explanation is not available. In pituitary basophilism, for example, marked negative nitrogen balance may be observed relatively early in the disease. Later, although the syndrome may be progressive, a negative nitrogen balance is difficult to demonstrate Perhaps adrenocorticotropic stimulation of the adrenals can evoke a preponderant secretion of one type of steroid, depending upon the conditions, type, and duration of the stress imposed Acute stress brings forth evidence of increased cortical activity with respect to both electrolyte and protein metabolism. In chronic stress, of type employed in these experiments, evidence of continuously increased activity of the salt-saving steroids is clear (It is to be recalled that in these experiments the salt-saving need comprises the initial and also the continuing stimulus for increased adrenocorticotropic activity) Metabolic evidence of increased activity of those steroids which affect protein metabolism is obvious initially but gradually disappears If, however, a new acute stress is suddenly superimposed upon the chronic one, for example, abrupt salt restriction in the fully acclimatized man working in the heat (Fig 2), or if the

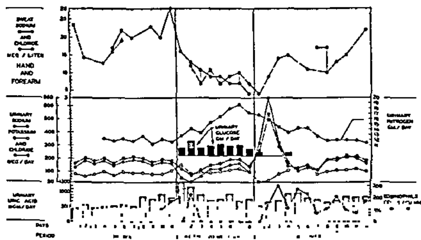
nism, the very one which must be stimulated to increased activity, if physiologic acclimatization to heat is to be accomplished.

On Full Acclimatization. When it was found that DCA interferes with the mechanism by which the process of acclimatization to heat proceeds, it seemed worth probing into the state of affairs which exists in the fully acclimatized man. DCA was again the experimental tool. Figure 5 illustrates the results. After 38 days of hard work and continuous residence in the heat, the subject was fully acclimatized. Before the administration of DCA (days 38 to 42) body weight was constant, nitrogen was in equilibrium, and concentration of sodium and chloride in the sweat was low—the characteristic status of full acclimatization. Injection of 3 5 mg. of DCA daily promptly produced a further fall of the sodium and chloride concentration of the sweat. An even greater effect was observed when the dose was increased to 7 mg. per day. While the sweat glands reflected the increased DCA activity throughout the period of injection, the renal tubules did not, akin to the rebound phenomenon (15) Nitrogen equilibrium was maintained in this period. The subject performed his daily task efficiently and with no untoward symptoms during the entire period of DCA administration.

Within 24 to 48 hours after DCA administration was discontinued, a dramatic change in both performance and metabolic pattern occurred The subject began to complain of dizziness and "light-headedness" in the erect position, which was relieved by recumbency Peripheral vasoconstriction was evidenced by marked pallor. The rectal temperature rose to levels that had not been recorded since full acclimatization had been established Muscular weakness, nausea, and "too hot" were important complaints He was physically unable to complete the usual amount of work, and the load was lightened

An interesting change in the metabolic pattern was occurring simultaneously (Fig. 5). The concentration of salt in the sweat rose abruptly to the preacclimatization level Despite decreasing body weight and urinary diuresis, the urinary nitrogen decreased. This pattern had also been observed when DCA had interfered with the process of acclimatization (Fig 4). By the fourth day after cessation of DCA administration, the metabolic pattern of reacclimatization was evident. Acclimatization then proceeded very rapidly. By

heat by injections of exogenous ACTH, the entire thesis of the mechanism of acclimatization would receive critical experimental support. Recently we (10a) have performed such experiments* and believe that the results constitute substantial confirmation of the prior deductions.



EFFECT OF ACTH UPON SWEAT Na AND Cl IN RELATION TO URINARY Na, Cl, K, GLUCOSE, NITROGEN, URIC ACID, AND EOSINOPHILS (A.M. p 23 NORMAL CONTROL)

Fig 7. Effect of adrenocorticotropic hormone upon sodium and chloride content of sweat in relation to changes in urinary sodium, chloride, potassium, nitrogen, glucose, and uric acid

Figure 6 demonstrates the effect of ACTH upon concentrations of sodium and chloride in sweat. Note that samples of sweat have been collected from two sites of the skin simultaneously. The effect of ACTH in decreasing the concentrations of sodium and chloride in sweat is similar in all respects to that observed when DCA is administered The conclusion seems justified that ACTH stimulates increased adrenal elaboration of salt-active corticosteroids. That it causes also increased production of androgenic corticosteroids is seen in the great increase observed in daily urinary excretion of 17-ketosteroids.

* We are indebted to Dr J R Mote, Armour Research Laboratories, Chicago, Illinois, for the purified adrenocorticotropic hormone used in these studies.

chronic stress is temporarily removed and the pituitary–adrenal mechanism is put at relative rest, then when the stress is suddenly reapplied, as in DCA administration and cessation in the fully acclimatized man working in the heat (Fig. 5), a negative nitrogen balance returns for a short period of time. It seems likely that acute

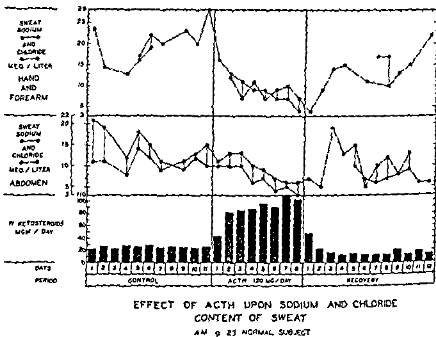


Fig 6. Effect of adrenocorticotropic hormone upon sodium and chloride content of sweat.

excitation of the pituitary–adrenal axis results in the liberation of excessive amounts of the several types of steroids which fall within its productive capacity, and that with increasing chronicity of the stimulus the steroid produced in increased amounts conforms more closely to the type which is required to satisfy the physiological demand, since it is this demand which constitutes the continuing stimulus.

Experiments with Purified Pituitary Adrenocorticotropic Hormone (ACTH) in Normal Individuals. From what has been either demonstrated or inferred above, it becomes clear that were one able to duplicate the metabolic pattern of acclimatization to

certain physiologic adjustments, which result, after a variable number of days of such exposure, in a vast improvement in his ability to perform a given task with relative ease and without untoward symptoms. This adaptive phenomenon has been called acclimatization to heat. Careful studies to determine differences in physiologic processes between the unacclimatized and the acclimatized man indicate that the major differences exhibited by the latter consist of an improved peripheral circulation and an enhanced ability to resist depletion of body salt. This second difference manifests itself mainly as a capacity to produce sweat which is much lower in its concentrations of sodium and chloride than was the case prior to acclimatization Since under conditions of work in the heat the major loss of body salt occurs via sweating, this adjustment assumes primary importance.

The mechanisms by which these adjustments are accomplished *during the process of acclimatization* have not been known. Extensive metabolic studies done in our laboratory in the past five years appear to establish the details of this mechanism. On the basis of the metabolic phenomena observed repeatedly, it is believed that in man the process of acclimatization to heat consists of an increased activity of pituitary adrenocorticotropic hormone and that the resulting enhancement of the production and liberation of adrenal cortical steroids is responsible for bringing about the physiologic adjustments characteristic of state of acclimatization. Under the conditions of our experiments, the need for the conservation of body salt constitutes the stimulus which "fires" the mechanism and which raises the level of activity of the pituitary–adrenal axis

References

1 Adolph, E F · Heat exchanges of man in the desert Am J Physiol *123*, 486, 1938

2 Asmussen, E · Cardiac output in rest and work in humid heat Am J Physiol *131*, 54, 1940

3. Bazett, H C : The effects of heat on blood volume and circulation J A. M. A. *111*, 1841, 1938

4 Bazett, H C, Sunderman, F W, Doupe, J, and Scott, J C. Climatic effects on the volume and composition of the blood in man Am J Physiol *129*, 69, 1940

4a Bethel, F. H, Neligh, R. B, and Conn, J W. Influence upon the peripheral blood of prolonged administration of pituitary adrenocorticotropic hormone in normal humans J. Lab & Clin. Med In press.

Figure 7 represents additional metabolic balance data obtained upon the same subject during the same experiment. The changes observed in the sweat are charted again (upper curve) in order that they be seen in proper temporal relationship with the other metabolic changes that occur during acute adrenocortical stimulation. It is of interest to observe first the renal effect of endogenously produced salt-active (desoxycorticosterone-like) steroids. A prompt decrease of urinary excretion of sodium and chloride occurs. Within 3 or 4 days, however, these values return to the base line despite continued administration of ACTH. This represents the same renal "rebound" which occurs during continued injections of DCA. The sweat, on the other hand, continues to reflect increased activity of salt-active corticosteroids for as long as the ACTH is administered Thus, it is evident that precisely the same effects upon urinary and sweat electrolytes are produced by either DCA or ACTH, and that the same pattern is observed to occur spontaneously during the process of acclimatization to heat. In addition, it is obvious that urinary excretion of electrolytes is a poor index of continued excessive activity of salt-active corticosteroids, while sweat electrolyte composition may prove to be a reliable one (7a).

Finally, attention must be focused upon the negative nitrogen balance (Fig. 7) which accompanies the changes in urinary and sweat electrolytes produced by administration of ACTH. This loss of body protein represents an activity of corticosteroids of the "11-oxysteroid" type which, too, have been produced in excessive amounts as the result of ACTH stimulation. Other evidence of increased "11-oxysteroid" activity is observed in the changes in urinary uric acid, in the occurrence of glycosuria, and in changes in the cellular elements of the peripheral blood These changes have been reported in detail in other connections (4a,10b,10c,23a).

Thus, it can now be stated that all of the metabolic features which we have observed to be characteristic of the process of acclimatization to heat, which features we have interpreted as indicating increased adrenocortical activity by virtue of increased production of pituitary adrenocorticotropic hormone, can be duplicated in normal humans given injections of a purified preparation of ACTH.

Summary

It is a well-established fact that man suddenly confronted with the necessity of performing work in the heat, is capable of making

with the method of preventing their fatal consequences . . . , 2d ed. London, T. Becket, 1771.
19 Mickelsen, O., and Keys, A.. The composition of sweat, with special reference to the vitamins J Biol Chem *149*, 479, 1943
20 Pitts, G C., Johnson, R E, and Consolazio, F C.· Work in heat as affected by intake of water, salt and glucose. Am. J. Physiol *142*, 253, 1944
21. Robinson, S, Dill, D B, Wilson, J W, and Nielsen, M : Adaptations of white men and Negroes to prolonged work in humid heat. Am J. Trop Med *21*, 261, 1941
22 Robinson, S, Turrell, E. S, Belding, A S, and Horvath, S M : Rapid acclimatization to work in hot climates Am J Physiol. *140*, 168, 1943
23 Robinson, S, Turrell, E. S, and Gerking, S D · Physiologically equivalent conditions of air temperature and humidity. Am J Physiol *143*, 21, 1945
23a Robinson, W. D, Conn, J W, Block, W. D, and Louis, L H · Role of the adrenal cortex in urate metabolism and in gout. J Lab & Clin Med *21*, 23, 1948
24 Sayers, G, and Sayers, M A Regulation of anterior pituitary activity during stress Proc Am Federation Clin Research *2*, 96, 1945
25 Scott, J C, Bazett, H C, and Mackie, G C Climatic effects on cardiac output and circulation in man Am J Physiol *129*, 102, 1940
26 Talbott, J H · Heat cramps Medicine *14*, 323, 1935
27. Talbott, J. H, Dill, D. B, Edwards, H V, Stumme, E H, and Consolazio, H V The ill effects of heat upon workmen J Indust Hyg & Toxicol *19*, 258, 1937
28 Taylor, H L, Henschel, A F, and Keys, A Cardiovascular adjustments of man in rest and work during exposure to dry heat Am J Physiol *139*, 583, 1943
29 Taylor, H L, Henschel, A, Mickelsen, O, and Keys, A The effect of the sodium chloride intake on the work performance of man during exposure to dry heat and experimental heat exhaustion Am J Physiol *140*, 439, 1944.
30 Thorn, G W : Effect of cortical hormone on renal excretion of electrolytes in normal subjects Proc Soc Exp Biol & Med *36*, 361, 1937
31 Weiner, J S An experimental study of heat collapse J Indust Hyg & Toxicol *20*, 389, 1938

5. Blagden, C.: Experiments and observations in an heated room. Philosophical Trans, Royal Soc., London, *65*, 111, 1775
6 Carleton, E. H, and Kammer, A G: Heat sickness Rocky Mountain M J *40*, 384, 1943.
7 Collings, G H, Shoudy, L. A, and Shaffer, F. E.. The clinical aspects of heat disease Indust Med. *12*, 728, 1943.
7a Conn, J. W.: Clinical implications of electrolyte composition of thermal sweat as an index of adrenal cortical function Arch. Int Med. In press
8 Conn, J. W, and Johnston, M W.: Salt economy of the sweat glands under conditions of hard work in a tropical climate. J Clin Investigation *23*, 933, 1944
9 Conn, J W., Johnston, M W, and Louis, L H.: Acclimatization to humid heat. A function of adrenal cortical activity J. Clin Investigation *25*, 912, 1946
10 Conn, J W, Johnston, M W, and Louis, L H: Relationship between salt intake and sweat salt concentration under conditions of hard work in humid heat Federation Proc *6*, 230, 1946
10a Conn, J W, Louis, L H, Johnston, M W, and Johnson, B S: Electrolyte content of thermal sweat as an index of adrenal cortical function J Clin Investigation *27*, 529, 1948
10b Conn, J W, Louis, L H, Johnston, M W, Johnson, B S, Blood, J., and Pinkham E: Metabolism of uric acid, glutathione and nitrogen, and excretion of '11-oxysteroids' and 17-ketosteroids during induction of diabetes in man with pituitary adrenocorticotropic hormone J Lab & Clin. Med *34*, 255, 1949
10c. Conn, J W, Louis, L H, and Wheeler, C E. Production of temporary diabetes mellitus in man with pituitary adrenocorticotropic hormone, relation to uric acid metabolism. J Lab & Clin Med. *33*, 651, 1948
11 Dill, D. B.. Life, Heat, and Altitude Cambridge, Harvard Univ Press, 1938
12 Dill, D B, Edwards, H T, Bauer, P S, and Levenson, E J Physical performance in relation to external temperature Arbeitsphysiol *4*, 508, 1931
13 Dill, D. B, Hall, F G, and Edwards, H T Changes in the composition of sweat during acclimatization to heat Am J Physiol, *123*, 412, 1938
14 Eichna, L W, Bean, W B, Ashe, W. F, and Nelson, N Performance in relation to environmental temperature Bull Johns Hopkins Hosp *76* 25, 1945
15 Heilman, J D, and Montgomery, E S: Heat disease A clinical and laboratory study J Indust Hyg & Toxicol *18*, 651, 1936
16 Jackson, R: A treatise on the fevers of Jamaica containing some hints on the means of preserving the health of soldiers in hot climates Philadelphia, R Campbell, 1795
17 Keeton, R. W, Hick, F H, Glickman, N, and Montgomery, M M The peripheral type of circulatory failure in experimental heat exhaustion The role of posture Tr Am Soc Heat & Vent Engin *46*, 157, 1940
18 Lind, J. An Essay on Diseases Incidental to Europeans in Hot Climates,

Modern Therapeutic Agents Used in Neurologic Conditions

H. HOUSTON MERRITT

Columbia University, and Neurological Institute, Presbyterian Hospital, New York, New York

The advances in methods of treatment of diseases of the nervous system have kept pace with those in other branches of internal medicine. The acute bacterial infections of the meninges can now be controlled with the sulfonamides or penicillin. Treatment with these agents will produce a cure in almost all of the patients with meningococcic infections and in a high percentage of those with the hitherto highly fatal meningitides due to the pneumococci, staphylococci, and streptococci. Great strides have been made in the treatment of the paroxysmal disorders of the nervous system, epilepsy, migraine, and Ménière's syndrome. Although our knowledge regarding the pathologic physiology of these conditions is still limited, we now have treatments which are effective in preventing the attacks in a high percentage of the cases.

In spite of these advances, there are still a large number of diseases of the nervous system for which our therapy is inadequate. Among these are the tubercular infections of the nervous system, viral diseases (acute anterior poliomyelitis, viral encephalomyelitis), demyelinizing diseases (multiple sclerosis, acute encephalomyelitis), heredodegenerative diseases, muscular atrophies, and vascular diseases of the nervous system In addition, we cannot claim that our method of treating the disabling forms of neurosyphilis (dementia paralytica, tabes dorsalis) has been perfected, nor that the surgical treatment of tumors of the brain is a satisfactory answer to that problem

The purpose of this article is to present in detail some of the newer forms of treatment, and to discuss briefly the status of our treatment of some of the more common diseases of the nervous system. No attempt will be made to cover all neurologic conditions.

an elevated temperature and pulse rate The diagnosis is confirmed by the results of a lumbar puncture The cerebrospinal fluid is cloudy or frankly purulent and contains several thousand polymorphonuclear leukocytes. The protein content is increased, and the sugar content decreased to less than 15 mg. per hundred cubic centimeters in most cases. Organisms can be seen in stained smears or cultures on appropriate media. Treatment should not be delayed until the organisms are identified by culture studies

Treatment with Sulfonamides. Sulfanilamide and many of its derivatives (sulfapyridine, sulfathiazole, sulfapyrazine, sulfamerazine, sulfadiazine) have given good results in the treatment of meningococcic meningitis (1). Sulfadiazine is most commonly used because of its high therapeutic index and relatively low toxicity. The dosages given here are for sulfadiazine. It should be remembered, however, that any of the other sulfonamides can be substituted, if desired. If a patient is unable to take or retain the medication when given by mouth, the first dose should be given parenterally; it can be injected intravenously in the form of the sodium salt dissolved in distilled water as a 5 per cent solution. The amount of the initial dose should be approximately 0 05 to 0.1 Gm of the drug per kilogram of body weight, followed by intravenous injection of one-half the initial dose every 8 hours until the drug can be taken by mouth Subsequent dosage should be 1 to 2 Gm. orally every 4 hours so that a blood concentration of approximately 15 mg. per hundred cubic centimeters is maintained. Therapy should be continued with full dosages for approximately 4 days after complete symptomatic recovery and in reduced dosage for an additional 3 or 4 days. Intrathecal injection of the drug has been recommended by some workers, in our opinion, this is dangerous and should not be used The value of specific antiserums has never been proved, and they should not be used in conjunction with the sulfonamides

There is some debate as to the advisability of frequent lumbar punctures; experience has shown, however, that they are not necessary. The initial lumbar puncture is essential to the diagnosis; subsequent punctures can be made at 24 to 48 hour intervals in order to follow the course of the infection.

Toxicity. Minor toxic symptoms, such as nausea and vomiting, which were frequent with some of the other sulfonamides, are fairly

Infections of the Nervous System

Great strides have been made in recent years in the treatment of infectious diseases of the nervous system. The results obtained with sulfonamides, penicillin, and other antibiotics are in striking contrast to those formerly obtained with serum therapy. It is gratifying to note that the use of intraspinal therapy is rapidly being discarded, although some workers insist that this form of therapy should still be used with the newer drugs.

With the sulfonamides and penicillin, uniformly good results can be obtained in the treatment of meningococcic meningitis and the highly fatal forms of bacterial meningitis; that is, pneumococcic, staphylococcic, and streptococcic meningitides, respond in a large number of cases to either penicillin or sulfonamide alone, or when used in combination These drugs do not have any effect on the tuberculous form of meningitis, but the course of the infection can be arrested in the majority of the cases and clinical cure effected in a small percentage by the administration of streptomycin.

THE MENINGITIDES

Meningococcic Meningitis

Neisseria meningitidis is found in the nasopharynx. The frequency of the carrier state increases when crowding occurs, especially during cold weather. The various serologic types differ as to the frequency with which they cause septic meningococcemia. Invasion of the subarachnoid and cerebral ventricles usually takes place abruptly, often without purpura or other evidence of invasion of the blood stream The optimal therapeutic results are obtained only when physicians are on the alert, and when the diagnosis is established soon after the onset of fever and headache. However, with present methods of treatment, a fair result is obtained in many cases not treated until symptoms have been present for many hours or even days. The death rate and the incidence of deafness and other permanent disability rises with every hour lost after the onset of symptoms. The cardinal signs and symptoms are· headache, vomiting, chills, and skin rash. On examination, the patient is lethargic or stuporous, there is stiffness of the neck, a positive Kernig's sign, and

practically none of the drug reaches the cerebrospinal fluid when it is administered by mouth or parenterally. In our opinion, this reasoning is fallacious, for the cure of the infection is not related to the amount of drug present in the cerebrospinal fluid. If penicillin is given intraspinally, the dose should not exceed 5,000 to 10,000 units at one injection, and the injections should not be repeated more than once every 12 to 24 hours. The danger of radiculitis, myelitis, and other complications (3) is too great to permit intraspinal injection of large amounts of any medication. It is unsafe to inject penicillin intracisternally or intraventricularly because convulsive seizures, coma, and death (3) may follow.

The drug should be given intramuscularly in dosages of 300,000 units every 8 hours. This dosage should be continued for 6 to 8 days after the disappearance of all clinical symptoms and after the spinal fluid has returned to normal.

Complications. Complications with the intramuscular use of penicillin are slight, and no serious ones have been reported. Rarely, the occurrence of an urticarial rash with severe pruritus may necessitate withdrawal of the drug The intraspinal use of penicillin may produce damage to the roots of the cauda equina or to the spinal cord, and for this reason it should be avoided.

Pneumococcic Meningitis

The signs and symptoms of pneumococcic meningitis resemble those of meningococcic meningitis, except that a septic focus at the site of origin of the meningitis is usually present in patients with this form of the disease Until the advent of the sulfonamides, the mortality rate of pneumococcic meningitis was well over 90 per cent. With the use of the sulfonamides and penicillin, the mortality rate has been reduced to approximately 25 per cent (1). Both drugs are administered as described above for meningococcic meningitis. Since this disease has such a high mortality rate, it is advisable to use both agents simultaneously in full doses. In addition to the treatment of the meningitis, the focus which gave rise to the meningeal infection should be treated surgically, if necessary

Staphylococcic and Streptococcic Meningitis

Staphylococcic and streptococcic meningitis should be treated with penicillin alone, as outlined above, or with a combination of

infrequent with sulfadiazine. Dermatitis may occur with any one of the sulfonamides, but treatment need not be stopped unless the rash is progressive and severe. The most severe toxic effects are those involving the blood and the urinary tract. Acute hemolytic anemia may occur during the first few days of treatment, and rapidly increase in severity. This is rare with sulfadiazine. Marked leukopenia and granulocytopenia may occur during or after the second week of treatment, and are quite common with sulfapyridine. Both of these complications necessitate immediate cessation of drug therapy and administration of large amounts of fluid and alkali, as well as use of transfusions or other measures, if indicated. If the infection is not cleared up completely at this time, treatment must be continued with one of the other sulfonamides or with penicillin.

Renal complications, hematuria, oliguria, anuria, and pains in the flank typical of renal colic, may result from precipitation of crystals of the drug, mostly in the acetylated form, with the formation of small calculi in the urinary tract. Gross hematuria, hemoglobinuria, and anuria are indications for stopping treatment immediately and for intravenous administrations of fluid and glucose. Alkali in the form of sodium bicarbonate can be given orally or in a sixth-molar solution of sodium lactate intravenously, in order to make the urine alkaline. If calculi are present, urethral catheterization may be necessary to establish drainage of urine from the pelvis. If renal complications occur before the disease has been cured penicillin treatment should be instituted

Febrile reactions may occur after the first week of treatment, and it may be difficult to determine whether the fever is due to the drug or to a relapse of the infection. The presence of a rash or other toxic manifestations of the drug favor the diagnosis of drug fever. Examination of the spinal fluid will establish with certainty the cause of the fever.

Penicillin Therapy. Penicillin may be used alone or in combination with the sulfonamides in the treatment of meningococcic meningitis (2) The results reported in the literature indicate that penicillin is not quite as effective as the sulfonamides. Penicillin should be used in cases refractory to the sulfonamides or when complications prevent the use of the sulfonamides. It has been claimed that maximum benefit cannot be obtained with penicillin unless it is given intraspinally. This claim is based on the fact that

spinal fluid findings are, however, characteristic and a presumptive diagnosis can be made when the typical abnormalities are present. These include: an increased pressure; a slightly cloudy or ground glass appearance to the fluid, with formation of a clot on standing; a moderate pleocytosis varying between 25 and 500 cells per cubic millimeter with lymphocytes as the predominating cell type; increased protein content; decreased sugar content with values in the range of 20 to 40 m. per 100 cc.; decreased chloride content; negative Wassermann test; and absence of growth when the fluid is inoculated on routine culture media.

Until recently, tuberculous meningitis terminated fatally in almost 100 per cent of the cases. It is now known that the course of the disease can be favorably influenced by the exhibition of streptomycin. Complete cure with or without permanent residuals has been reported in a number of cases, and prolongation of life for a number of months can be obtained in the majority of the cases. Treatment with streptomycin should be started early and continued for several months. Daily therapy should include 2 to 3 Gm. intramuscularly and 100 to 200 mg. intraspinally.

CAVERNOUS SINUS THROMBOSIS

This condition was invariably fatal until the advent of the newer forms of treatment. Isolated cures began to be reported with the use of the sulfonamides, and some of these patients were treated with a combination of sulfonamides and heparin. The frequency of massive hemorrhages, particularly into the brain, makes the use of heparin inadvisable. There is no evidence that its use increases the incidence of cures. When penicillin became available, it was tried; administered intramuscularly in doses of 300,000 units every 8 hours, it has resulted in the cure of most of these cases (5)

EPIDURAL ABSCESS

Metastatic infection of the epidural fat may occur as a result of staphylococcic infections of the skin or other structures of the body. The symptoms are radicular pain in the thoracic, lumbar, or cervical region, with rapidly progressing weakness of the legs and disturbance of bladder control Continued compression of the cord

penicillin and the sulfonamides. Very good results have been obtained with the use of penicillin alone by the intramuscular route (2).

Influenzal Meningitis

Meningitis due to Hemophilus influenzae occurs most frequently in infants or young children Prior to the introduction of the sulfonamides, the mortality rate was over 90 per cent. Recovery rates as high as 50 per cent have been reported with use of the sulfonamides alone, but the best results are obtained when sulfonamides are used in combination with streptomycin and type specific rabbit antibody serum Sulfadiazine should be given in dosages sufficient to maintain a blood level of 8 to 12 mg per hundred cubic centimeters, and continued for 2 weeks after apparent clinical cure, as determined by the general condition of the patient and the results of examination of the cerebrospinal fluid, particularly the bacterial and sugar content. Type b H. influenzae rabbit antiserum is given intramuscularly or intravenously as soon as the diagnosis is established The initial dose should be in the range of 75 to 150 mg. of antibody nitrogen. Subsequent dosages are determined by assaying each day varying dilutions of the patient's own serum against a subculture of the organisms recovered from his cerebrospinal fluid Total dosages in the series treated by Smith and associates (4) averaged about 200 to 250 mg The mortality rate in their series of 28 cases was 7 per cent Streptomycin is given intramuscularly in doses of 0 15 to 0 3 Gm. every 3 hours.

Tuberculous Meningitis

Tuberculous meningitis is always secondary to tuberculosis elsewhere in the body. The primary focus of the infection is most commonly in the lungs, but it may be in the lymph glands, bones, gastrointestinal tract, or practically any other organ in the body The onset of meningeal symptoms may be coincidental with signs of acute miliary dissemination or there may be clinical evidence of activity in the primary focus, but not infrequently the meningitis may be the only manifestation of activity of the disease

The diagnosis of tuberculous meningitis can be made only by recovering the organism from the cerebrospinal fluid. The cerebro-

with the trivalent arsenicals and with bismuth. In addition to these compounds, neurosyphilis was treated with tryparsamide and fever therapy.

[illegible] such as mapharsen, for 20 to 30 weeks, followed by weekly injections of bismuth for 10 to 15 weeks. If symptomatic or serologic improvement was not obtained, the treatment was changed to either tryparsamide or fever therapy. Weekly injections of tryparsamide were given for 30 to 40 weeks and fever therapy resorted to if good results were not obtained. Because of the danger of amblyopia in patients treated with tryparsamide, many workers preferred fever therapy in these types of neurosyphilis if good results were not obtained with trivalent arsenicals and bismuth. Some even felt that it was wise to use fever therapy as the initial treatment, and to follow this with trivalent arsenicals and bismuth injections. A combination of these various methods led to the arrest of the process and a reversal of the cerebrospinal fluid changes to normal in practically all the cases.

Dementia paralytica was treated with fever therapy, either by inoculation malaria or fever cabinet, followed by trivalent or pentavalent arsenicals at weekly intervals for a period of 2 to 3 years. Approximately one-third of the patients so treated recovered, approximately another one-third were greatly improved, and about one-third were not benefited by treatment.

Neurosyphilologists have long realized the need for a more efficient, less time consuming, less expensive, and less dangerous treatment than those that have been used in the past. The preliminary reports of the beneficial action of penicillin on primary and secondary syphilis led to the hope that penicillin would be the answer to the problem. Unfortunately, at the time that this is written we are not able to evaluate accurately the role of penicillin in the treatment of syphilis of the nervous system. Too few cases have been treated, and these cases have not been followed for a sufficiently long interval to draw definite conclusions. In the small series of cases that have been reported (6), penicillin has been effective in reducing the spinal fluid abnormalities in meningeal and asymptomatic neurosyphilis, and it is effective in relieving the symptoms

or involvement of the blood supply may result in a complete, flaccid paraplegia. Epidural abscess should be suspected in all patients with rapidly developing spinal cord symptoms following upon furunculosis or other obvious pygenic infections due to Staphylococcus. Early diagnosis and treatment is imperative, to prevent irreparable damage to the spinal cord or spread of the infection to the leptomeninges. The diagnosis in a clinically suspicious case is established by the results of lumbar puncture. The typical findings are: (*1*) complete or incomplete spinal subarachnoid block; (*2*) cloudy cerebrospinal fluid, containing a moderate or large number of cells, predominantly polymorphonuclear leukocytes; (*3*) normal sugar content. A very low sugar content or the presence of bacteria indicates that the infection has spread into the pia-arachnoid. In performing the lumbar puncture, the needle should be inserted slowly and aspiration with a syringe done at frequent intervals as the epidural space is approached It may thus be possible, by aspirating pus, to prove the presence of an epidural abscess before the subarachnoid space is entered. Theoretically, it is possible, if these precautions are not observed, to produce an actual meningitis by introducing pus into the subarachnoid space.

Chemotherapy and surgical drainage of the abscess is the treatment of choice. If laminectomy is performed and adequate drainage is established before paraplegia has developed, complete recovery can be expected. If drainage is delayed until after paraplegia has developed, the degree of recovery will depend chiefly upon the nature of the damage to the cord If the function of the cord is disturbed by pressure, good results may be expected Poor results are usually due to necrosis of the cord secondary to thrombosis of its blood supply. Penicillin should be administered intramuscularly in doses of 300,000 units every 8 hours before the operation, and for a week or more postoperatively.

SYPHILIS OF THE CENTRAL NERVOUS SYSTEM

The treatment of neurosyphilis has been greatly simplified in recent years. Before the introduction of penicillin, the treatment of syphilis in general, and syphilis of the central nervous system in particular, were cumbersome and attended with considerable risk Syphilis of the body, other than the nervous system, was treated

into two groups—symptomatic and idiopathic epilepsy—but this concept of two types of epilepsy is a fallacious one. It is erroneous to state that the seizures of patients with symptomatic epilepsy are due to the organic lesion in the central nervous system. This lesion is constantly present but the fits are irregular in occurrence. Furthermore, we are not in a position to state that a brain lesion is not present in the patient with so-called idiopathic epilepsy. We can only say that our present methods of examination fail to reveal such a lesion

Epileptic attacks may be divided roughly into three groups. Some patients may have only one type of seizure, but it is rather common for the afflicted individual to suffer with two, or even three types of spells. The three types of attacks are. (*1*) petit mal; (*2*) grand mal (including jacksonian); (*3*) psychic equivalent or psychomotor attacks

Petit mal attacks, which are characteristically a disease of childhood, are accompanied by transient clouding of consciousness lasting for only a few seconds, with or without minor movements of the head, eyes, and extremities, and loss of muscular tone The phenomena which occur in a grand mal attack may be quite varied Characteristically, these attacks are ushered in by a warning (aura) and are followed by a sudden loss of consciousness with tonic–clonic spasms of the musculature, with or without urinary and fecal incontinence Psychic equivalent, or psychomotor attacks, are terms used to describe a heterogeneous group of epileptiform disturbances which do not conform to the classic grand mal or petit mal types of seizure The milder psychomotor attacks are often confused with petit mal attacks, but they differ from the latter in that the duration of the period of mental cloudiness is greater and the range of muscular movements is much more widespread They differ from the grand mal attacks in that the patient does not fall to the ground in a tonic–clonic seizure, with complete loss of consciousness. In the severer form of psychic equivalents, the patient may be in a clouded state for many hours and perform acts of which he is entirely unaware

Some understanding of the mechanism involved in the causation of convulsive seizures has been gained by an analysis of the electrical potentials of the cerebral cortex, as recorded on the electroencephalogram. It has been shown by Gibbs and Lennox that there

in the former type. Beneficial effects on dementia paralytica (4,6) have been reported by a few observers, but their statistics are complicated by the fact that in many instances the penicillin treatment was combined with arsenicals or with fever therapy. In addition, there has been no uniformity in the route of administration of the drug or the total dosage used. Some workers advocate the injection of penicillin intraspinally, and others even go so far as to advise injection intracisternally. Our remarks on this subject have been recorded above, but we repeat: we do not think it advisable to give any drug intraspinally when it can be avoided; we would therefore not be in favor of intraspinal injection of penicillin and would be absolutely against its use intracisternally. At the present time it can be stated that penicillin is equally as effective as the former methods of therapy in the treatment of all forms of syphilis of the nervous system. Meningeal, vascular, and tabetic forms of neurosyphilis can be treated with penicillin alone. Although the results of penicillin therapy in dementia paralytica are excellent, prudence dictates that the use of penicillin should be combined with fever therapy in the treatment of this malignant form of neurosyphilis.

The optimum total dosage and the duration of therapy have not been clearly worked out. It is probably wise to err on the side of overtreatment and give 8 to 12 millions of units of penicillin in a period of 14 days to patients with the less malignant forms of neurosyphilis. *Dementia paralytica should be treated with 10 to 15 millions of units of penicillin in a period of 14 to 21 days*, combined with fever therapy of malaria or artificial fever cabinet

Paroxysmal Diseases of the Nervous System

EPILEPSY

Epilepsy is not a disease entity but a symptom complex characterized by the periodic occurrence of transient disturbance of consciousness, with or without convulsive movements Such a definition is inadequate, since the disturbance of consciousness may be so slight that it cannot be detected by superficial observation, even though convulsive phenomena may be present. On the other hand, recurrent attacks of loss of consciousness may occur in conditions not included in the category of epilepsy Epilepsy has been divided

drug has stimulated interest in the treatment of patients with convulsive seizures, and has led to a systematic search for a more efficient type of therapy.

The treatment of patients with convulsive seizures can be divided into three categories: (*1*) elimination of the important factors in the causation or precipitation of attacks; (*2*) general mental and physical hygiene; and (*3*) medical therapy directed toward raising the convulsive threshold, and thus preventing attacks.

Treatment of Underlying Physiologic or Structural Abnormalities This means elimination of any abnormal factor discovered in the thorough examination of the patient It would include surgical removal of operable tumors of the brain, evacuation of brain abscess, and treatment of infections or endocrine abnormalities, such as hypoparathyroidism or hyperinsulinism It is also important to correct any physical defects The question of the advisability of removing scar tissue resulting from traumatic or vascular injuries to the brain will be discussed more fully later.

Mental and Physical Hygiene. It is only rarely that the elimination of causative factors will result in the disappearance of attacks; in the vast majority of patients, control of the attacks require, in addition, physical and mental hygiene and the administration of anticonvulsive remedies It must be remembered that the period of treatment in the majority of patients is measured in terms of years or a lifetime. Patients must be encouraged to use all of their resources to overcome their feelings of inferiority and self-consciousness resulting from the attacks Adults should be assisted in obtaining some productive work which will occupy their time and perhaps give them some remuneration. Children should be kept in school unless the frequency of attacks unduly disturbs the routine of the classroom, or unless mental deterioration requires special facilities. A long-continued schedule of psychotherapy is of value for some patients, aiding them to adjust to their difficulties, but it cannot be expected to have any significant effect on the frequency of the attacks. Education of other members of the family in regard to their attitude toward the patient's illness is of great importance Excessive attention and oversolicitousness should be eliminated, and the family should not be allowed to make a chronic invalid of the patient

The patient's physical activity should be regulated so that there

are disturbances in the electrical activity of the cortex which are characteristic for each of the three forms of convulsive seizures described above. The electroencephalogram is not only of value in recording the changes that occur coincidentally with attacks, but also in registering characteristic short bursts of abnormal activity in the interval between attacks. A vast majority of patients with seizures will show a paroxysmal abnormality of the electroencephalogram in the interval between attacks The finding of an

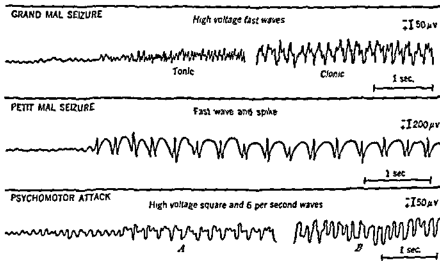


Fig 1 Example of electroencephalographic records of patients before and during epileptic seizures The first portion of each record shows the normal cortical activity for each patient; the second shows the abnormality characteristic for the type of attack, as labeled Courtesy of Dr F A Gibbs.

entirely normal electroencephalogram does not, however, exclude the diagnosis of epilepsy, since about 15 per cent of patients with epilepsy will have a normal record when only one tracing is taken in the seizure-free interval Examples of the electrical activity of the cortex in various types of convulsive seizure are shown in Figure 1

Treatment

With the introduction of phenytoin sodium in 1938, the treatment of epilepsy entered a new era The success obtained with this

on the nature of the attacks and the age of the patient. It must be remembered that if satisfactory results are not obtained with one of these drugs the others should be tried. In some patients a combination of two or more of the drugs will yield much better results than the use of one alone The therapeutic dose for any given patient must be determined by trial and error. It is not infrequently found that a drug has been discarded as useless, whereas in reality a slightly increased dosage would have led to a complete disappearance of all the attacks. On the other hand, it is inadvisable to push a drug to the point where the patient is so dull and stupid that he is more incapacitated by the toxic effects of the drug than by the seizures. The question as to whether drugs may be the cause of mental deterioration in patients with seizures has never been adequately settled; the evidence at present indicates that, with rare exceptions, the mentality of the patient is unchanged by medical treatment, unless the drugs are given to the point of intoxication. Not infrequently, the complete freedom from seizures following drug therapy will be accompanied by an improvement in the mental faculties

Indications for Use of Specific Drugs. For patients with infrequent grand mal seizures, phenobarbital can be tried first because of its high therapeutic index and its relatively low toxicity. If satisfactory results are not obtained, phenytoin sodium should be tried A combination of phenobarbital and phenytoin sodium is often more effective than either of the drugs used alone When these drugs are used in combination, a full therapeutic dose of each drug must be given In patients with frequent grand mal seizures or attacks of the psychic equivalent or psychomotor type, phenytoin sodium is the drug of choice Tridione is the drug of choice in the treatment of children with frequent petit mal attacks

Dosages to Be Used. *Phenobarbital* For the average adult the initial dose of phenobarbital should be 1½ grains daily This can be given at bedtime. After a trial period of 2 weeks or as long as is necessary to determine whether this dose is effective, increases can be made in the dosage until the patient is taking as much as 4½ to 6 grains per day. If this amount of the drug is not sufficient to control the seizures, a further increase will probably be valueless In children, the dose of phenobarbital should be in proportion to weight; however, it has been found that children are able to tolerate

is a set time for eating and sleeping and regular exercise every day. This exercise should be of a moderate nature, and the patient should not participate in competitive sports to the point of exhaustion. Meals should be wholesome and simple, with the proper amount of carbohydrates and proteins and an abundance of fresh fruits and vegetables. Alcoholic beverages are to be absolutely avoided. Bowels can be regulated by training and, if necessary, by the judicious use of mild laxatives. The patient should have a set time for retiring and arising, and should not be allowed to stay in bed after the other members of the household have arisen. Activities such as parties, dancing, movies, and so on should be encouraged. Swimming, horseback riding, and other dangerous sports can be permitted when there are proper safeguards The risk involved in such activities is justified in most instances in order to prevent the development of chronic invalidism Activities which endanger the lives of others, such as automobile driving, should be prohibited. Commitment of the patient to an institution is not desirable unless mental deterioration or unduly violent or frequent attacks which cannot be controlled by treatment make it necessary On the other hand, definitely deteriorated, destructive, or dangerous patients should not be kept at home and allowed to ruin the lives of other members of the family.

Medical Therapy The efforts directed toward the social and mental hygiene of the patient are of great importance, but the success of such measures depend to a large extent upon the ability of the physician to prevent the occurrence of seizures There are effective measures at hand for the prevention of seizures and they should be given an adequate, thorough trial in each individual patient The most effective method for control of seizures is use of the anticonvulsive drugs phenobarbital, phenytoin sodium (dilantin sodium), mesantoin, tridione, and bromides. Other forms of treatment such as the ketogenic diet and dehydration, have been discarded by most physicians The ketogenic diet has been found to be effective in controlling or reducing the frequency of seizures in children, but the difficulties of administering the diet are great. Furthermore, it has been shown that the patients who are helped by the ketogenic diet are equally benefited by the anticonvulsive drugs The same holds true of the dehydration treatment

Which drugs should be used in a given case depends somewhat

drug is decidedly alkaline; the gastric upsets which it may cause can be prevented by giving the drug along with the meal or with some food

The toxic effects of phenytoin sodium are different from those of phenobarbital, nervousness or sleeplessness, rather than drowsiness, being the more common early symptom Other symptoms of toxicity are gastric distress, nausea and vomiting, unsteadiness of gait, hypertrophy of the gums, dermatitis, and psychotic symptoms

The minor toxic manifestations of nervousness and a slightly unsteady gait are frequently transient; they appear during the first few days of therapy and disappear with its continuation or when the dosage is temporarily reduced High enough dosages can produce nystagmus and ataxia in practically all patients A few adults will tolerate as much as 9 to 12 grains (0 6–0 8 Gm.), but as a rule nystagmus and ataxia develop when the dose is increased beyond 7½ grains (0 5 Gm). Their appearance calls for a temporary or permanent reduction of the dosage. If the reduced dose is not effective in controlling the seizures and attempts to increase the dose again bring on these signs of toxicity, a combination of phenytoin sodium and phenobarbital or bromides should be tried.

Gastric discomfort, nausea, and vomiting may be controlled by giving the drug with a little bicarbonate of soda or at mealtime. Dermatitis occurs within 2 weeks of instituting therapy in approximately 5 to 10 per cent of the patients, is usually of a scarlatiniform or morbiliform nature, and is accompanied by fever. The rash usually disappears within a few days after withdrawal of the drug If the rash recurs when treatment is reinstituted or an exfoliative dermatitis develops, further use of the medicine is precluded

One of the troublesome toxic effects of the drug is hypertrophy of the gums This is most common in children, and varies from a slight swelling of the gums to a marked hyperplasia with an almost total covering of the teeth The hyperplastic tissue is usually firm, without any tendency to bleeding, and is unrelated to any disturbance in the absorption or utilization of vitamin C. The swelling can be retarded by massaging the gums daily. Any excessive growth of the gum tissue can be excised by the dentist.

The development of psychotic symptoms in patients under phenytoin sodium therapy is rare, and usually it is impossible to deter-

and require almost as large a dose as adults. It is therefore advisable to give children over 6 or 7 years of age the minimum dose of 1½ grains per day. Toxic side effects of phenobarbital are rarely serious An allergic rash of a scarlatiniform or morbilliform nature develops in only a fraction of 1 per cent of the patients who receive the drug. The occurrence of a rash calls for temporary withdrawal of the drug If the rash recurs on subsequent administration, further use is contraindicated because of the danger of exfoliative dermatitis Drowsiness and lethargy develop in about 5 to 10 per cent of the patients who are given phenobarbital. In some patients, this will disappear with continued therapy; in others, the persistence of the symptoms may prevent further use of the drug. Ataxia of the gait, tremors of the extremities, and nystagmus can develop in practically all patients if given large dosages of phenobarbital; they are rare, however, with dosages of less than 3 to 4 grains per day.

Phenytoin Sodium. (Dilantin Sodium). Its chemical formula is 5,5-diphenylglycolylurea The value of this drug in the therapy of convulsive seizures was first reported in 1938 *(7)*. It is particularly valuable in the treatment of psychomotor and grand mal attacks The advantage of this drug over phenobarbital and the bromides lies in the fact that it has very little or no hypnotic activity Regulation of the dosage is more difficult, however, and minor toxic symptoms are more frequent (8). The toxic effects are not serious, and it is almost impossible for a patient to take a fatal dose of the medicine.

The principle of administration of phenytoin sodium is similar to that of phenobarbital. namely, to establish and maintain a reservoir of the drug sufficient to control the seizures. In the average adult, the initial dose should be 1½ grains (0 1 Gm) 3 times daily If any seizures occur after 2 weeks of this dosage, it should be increased to 6 grains (0 4 Gm) daily Further increases in the dosage should be by increments of 1½ grains (0 1 Gm) until the maximum dose of 9 grains (0 6 Gm) daily is reached In the majority of adults, 6 grains (0 4 Gm) is the optimum dose In children over 12 or 14 years old, the average dose is 4½ to 6 grains (0 3–0.4 Gm.) and in younger children 3 to 4½ grains (0 2–0 3 Gm.). The medicine can be given in divided doses spread out through the day or it can be given all in one dose at bedtime The

doses for children according to size In the absence of signs of toxicity, this dose can be increased to a maximum of 30 grains (2 Gm.) 3 times daily. The chloride intake must be kept at an adequate level, to prevent undue replacement of chloride ion in the body fluid by the bromide. Facilities for the determination of the bromide content of the serum should be available. The effective level may be as low as 100 mg. per hundred cubic centimeters in some patients, whereas 300 mg may not be effective in others. Toxic symptoms usually develop with a concentration of 150 mg or more. The chief objections to the use of bromides lie in the frequent development of skin rash and their reputed tendency to produce mental dullness

Phenytoin Sodium with Mesantoin, Phenobarbital, or Bromides. Since phenytoin sodium has very little sedative effect, it is particularly adapted for use in combination with phenobarbital, mesantoin or bromides.

These combinations (9) can be used when one of the drugs is not effective in controlling the seizures or when the effective dose of phenytoin sodium alone produces toxic symptoms. The dosage of the combination must be worked out according to the tolerance of each patient. In the more resistant cases, 3 to 5 doses a day of a combination of 1½ grains (0 1 Gm.) of phenytoin sodium with ½ grain (0 03 Gm) of phenobarbital, 0.1 to 0 2 Gm. of mesantoin, or 15 grains (1 Gm.) sodium bromide, are usually required.

Other Forms of Therapy. *Ketogenic Diet.* It has been shown that a shift in the acid–base balance of the body fluid to the acid side tends to prevent seizures This can be accomplished by the ingestion of acid or acid-forming salts, but the use of such substances for long periods is not desirable A satisfactory acidosis can be produced by a diet which contains an excess of fats over carbohydrates. Good results in the control of seizures by this diet have been reported by numerous observers As stated before, however, patients that respond to this form of therapy usually also respond to phenobarbital or phenytoin sodium therapy, and since the administration of these drugs is a simpler procedure than the establishment of ketosis, the ketogenic diet has fallen into disuse.

Glutamic Acid The use of glutamic acid as an adjuvant in the treatment of convulsive seizures was suggested by Price, Waelsch, and Putnam in 1943 (10) These authors found that when glutamic

mine whether these symptoms are related to the use of the drug or not. In such cases, a change in the type of treatment should be tried.

Mesantoin (3-Methyl-5-phenylethylhydantoin). This hydantoin is occasionally more effective in the treatment of patients with grand mal seizures than are other drugs. Relatively large dosages are required (0 4 to 1 0 Gm. daily). The drug can be administered in combination with phenytoin sodium. The toxic side effects of mesantoin are similar to those of phenytoin sodium and include ataxia and allergic dermatitis. In addition it has more sedative effects than phenytoin and a few cases of fatal blood dyscrasia have occurred. Hypertrophic gingivitis does not develop.

Tridione (3,5,5-Trimethyloxazolidine-2,4-dione). Preliminary experiments (11) with this compound have given encouraging results in the treatment of petit mal attacks. Since the drug is of no value in the control of grand mal or psychomotor seizures, patients subject to one of the latter types of seizures as well as petit mal should be given phenytoin sodium or phenobarbital along with the tridione

The dosage of tridione for the treatment of petit mal varies from 0 3 to 2 0 Gm daily, starting with 0 3 Gm. and gradually increasing the dose until the seizures are controlled or evidence of toxicity appears Among the minor toxic symptoms are skin rashes, which require a cessation of the treatment, and visual symptoms due to an unusual sensitivity to light The latter is apt to develop in adolescent or adult patients, and is uncommon in young children. The photophobia is not accompanied by any change in visual acuity and disappears when the medicine is discontinued. More serious side effects include pancytopenia and nephrosis. In order to prevent, if possible, the development of a severe blood dyscrasia, complete blood counts should be made every 30 days The drug should be discontinued if there is any significant anemia or if the granulocytes are reduced below 3,000 per cubic millimeter

Bromides These were formerly the main therapeutic agent in epilepsy but they have now been replaced almost entirely by phenobarbital and phenytoin sodium Occasionally, however, they are effective when other forms of therapy fail Although any of the salts may be used, the drug is most commonly given as the sodium or potassium salt, in tablets or aqueous solution The average dose for an adult is 15 grains (1 Gm) 3 times daily, with proportionate

a trigger mechanism for the seizures. Good results following such excisions have been obtained by a number of neurosurgeons Treatment should be limited to patients with focal attacks who do not respond to medical therapy. In addition, the operation should be performed only by such neurosurgeons as have adequate facilities for locating the lesion Medical treatment must be used after operation. It is difficult to evaluate the results that have been obtained by surgery in these patients, since they are treated with anticonvulsants after the operation.

The excision of isolated foci of abnormal electric activity, as shown by the electroencephalogram, is still in the experimental stage. It cannot be advised as yet, for it is possible that the excision of such abnormal foci will only result in shifting the abnormality to another region of the cortex.

Operations other than on the central nervous system are not advisable unless indicated for reasons apart from the occurrence of convulsive seizures. Removal of the cervical sympathetics or portions of the large intestine, operation on the sinuses, and the like have no effect on the ultimate course of the seizures. Removal of pancreatic tumors is of course necessary when attacks are definitely proved to be related to hyperinsulinism Removal of the carotid sinus may be of benefit in patients with carotid sinus syncope.

MIGRAINE

Migraine is a symptom complex characterized by recurrent attacks of headache, sometimes hemicranial in type, associated with visual and gastrointestinal symptoms There is a tendency to familial occurrence. Rarely, the headaches may occur without the other symptoms; in such cases the diagnosis is insecure unless there is a history of typical migraine attacks in other members of the family The headaches may be generalized or localized at the front, back, or one side of the head They may be preceded by scintillating scotomas, with or without hemianopia The headaches gradually increase in intensity, usually reaching a climax within a few hours. Gastrointestinal symptoms, nausea, and vomiting are almost always present The duration of an attack varies from a few hours to a day or more, and their frequency from once a day to once or twice a year Women are more susceptible than men and attacks in women are often associated with the menses. The cause of mi-

acid was used by itself it had no effect on grand mal seizures, but used in combination with phenytoin sodium or phenobarbital it tended to reduce the frequency of petit mal or psychomotor seizures in children; it was also effective in controlling behavior difficulties in such children. Very large doses of glumatic acid, 8 to 20 Gm per day, are required.

Status Epilepticus. Patients who are subject to seizures may have attacks so frequently that they do not recover from the coma produced by one attack before the next attack supervenes. The patient remains in coma for 12 to 24 hours, during which time there may be many convulsive seizures. The attacks may cease spontaneously and the patient recover consciousness after a period of 24 to 48 hours, or death may occur as the result of the repeated attacks. The likelihood of the latter eventually is so great that vigorous therapeutic methods aimed at terminating the seizures are justified. Good results can sometimes be obtained by anesthetizing the patient with one of the volatile anesthetics such as chloroform or ether, but termination of the seizures is more certain with the injection of sodium phenobarbital or paraldehyde, intravenously, and there is less risk of pulmonary complications. It is important that a large dose be given at the first injection, for best results are obtained when the full amount is given in one rather than in divided doses For adults, 6 to 12 grains (0 4–0 8 Gm.) of sodium phenobarbital dissolved in distilled water, or 3 to 6 cc. of paraldehyde, should be injected intravenously. The dosage for children should be from 3 to 6 grains (0 2–0.4 Gm.) of sodium phenobarbital. or 2 to 4 cc of paraldehyde, according to the size of the child

Surgical Treatment. Whenever convulsive seizures are associated with a surgically removable lesion of the brain, such as tumor or abscess, removal of the lesion is indicated. It must be remembered, however, that the relief of convulsive seizures will result in only about 50 per cent of patients with meningioma of the brain and in a much smaller percentage of patients with glioma or abscess of the brain In such cases, further treatment with drugs is necessary.

In addition to the removal of expanding lesions, surgery has been advocated for the removal of cortical scars secondary to cerebral trauma, vascular lesions, and birth injuries, on the assumption that such scars produce irritation of the neighboring cortex and act as

intravenously and follow this in 30 to 45 minutes, if relief is not obtained, with 0.25 mg. subcutaneously. Atropine sulfate, $^1/_{150}$ to $^1/_{100}$ grain by mouth or subcutaneously, helps to relieve the associated nausea and vomiting

Ergotamine Tartrate, Orally Taken by mouth, the drug is effective in a much smaller percentage of patients than by the parenteral route, but it should be tried in all cases From 3 to 5 mg. should be given immediately at the onset of the attack, followed by 1 to 2 mg. every half-hour until relief is obtained or until a maximum of 12 mg. is taken.

Contraindications. Because of its effect on the cardiovascular system, ergotamine tartrate is contraindicated in patients with arteriosclerosis, hypertension, or coronary disease. Its use is also contraindicated in patients with acute infections, with hepatic disease, or with avitaminoses, particularly vitamin C deficiency. Pregnancy is not an absolute contraindication to the use of ergotamine tartrate, but it should be avoided at this time if possible.

Complications Frequent usage over a long period of time carries with it the dangers of ergotism, so that no more than 0.5 mg. parenterally or 12 mg by mouth should be given in 12 hours. Ergotism has not been reported from the oral use of the drug; it is quite rare when administered intravenously even when given frequently over a long period of time The development of such signs and symptoms as paresthesia, numbness, coldness, and absence of vascular pulsations in the extremities prohibits further use of the drug

Untoward symptoms of minor significance, such as nausea and vomiting, occur in approximately two-thirds of the patients when the medicine is injected intravenously These symptoms can be alleviated by atropine Muscular pains, which develop in a small percentage of patients after treatment, are alleviated by calcium gluconate Transient paresthesias, weakness, substernal oppression, and drowsiness may occasionally follow the administration of the drug

Other ergot derivatives have been used in place of ergotamine tartrate Lennox reported that ergonovine (13) was less likely to produce nausea and vomiting Oral use of ergonovine produced relief of symptoms in a slightly higher percentage of patients than ergotamine tartrate, but parenteral injection was effective in a smaller

graine headaches is as yet unknown, but it has been shown that onset of the headache is accompanied by dilatation of the extracranial vessels and it is possible that there is also dilatation of the intracranial vessels.

Treatment of migraine, once the diagnosis is established by a careful history, examination, and laboratory studies, resolves itself into two phases. (*1*) general treatment of the patient, and (*2*) treatment of the individual attacks.

General Treatment. This is of paramount importance in preventing or decreasing the number of attacks. Physical defects should be corrected, allergic factors should be avoided, and special attention must be paid to the patient's psychologic difficulties. It is a common observation that the frequency of the attacks is markedly affected by unpleasant or difficult situations Sources of conflict should be eliminated by psychotherapy, change of environment, and regulation of the regime

In the majority of cases the common analgesic drugs have little or no effect on the headache Morphia and the other narcotics may produce relief but their use should be avoided for obvious reasons Prompt relief of symptoms can be obtained by the parenteral injection of ergotamine tartrate or dihydroergotamine methane sulfonate (D.H E.) early in the attacks These drugs can be administered by mouth but are much less effective

Treatment of Individual Attacks. *Ergotamine Tartrate (Gynergen) Parenterally* As soon after the onset of the headache as possible 0 5 mg (1 cc of a 1 2,000 solution) of the drug should be injected Its effect is to contract dilated cranial vessels Wolff (12) has shown that prolonged dilatation of the vessels during an attack is accompanied by an edema of the vessel wall which will interfere with the contracting effect of the ergotamine tartrate Therefore, if the treatment is to be effective it must be given in the attack, before this edema develops When given intravenously, the relief from headache is quicker, results are more certain, and there is no pain at the site of the injection On the other hand, subcutaneous injection is less apt to be followed by nausea, vomiting, and muscle pains The amount of ergotamine tartrate required to relieve an attack is variable, and must be determined for each patient A suitable procedure is to inject 0.25 mg (0 5 cc of a 1 2 000 solution)

has been suggested that the attacks are due to an acute hydrops of the labyrinth On this basis, a dehydrating, acid-forming diet was introduced as a therapeutic measure by Furstenburg and associates (15). More recently, Talbott and co-workers (16) have shown that there is a relatively high potassium content in the serum of these patients This they consider as evidence of a depletion of this element in the tissue cells (similar to the high level of serum calcium in regard to its storage in the bones). On the basis of this theory, the administration of potassium by mouth should restore the potassium in the tissues to a normal level. Atkinson (17) has postulated that the attacks of acute vertigo in patients with Ménière's syndrome may be due to one of two causes: (*1*) histamine sensitivity, in which there is a primary vasodilatation, and (*2*) primary vasospasms, with secondary vasodilatation His treatment of the first group consists of desensitization to histamine, while patients in the second group are treated with vasodilator drugs, such as nicotinic acid.

Since there is no satisfactory explanation of the pathologic physiology of the attacks in Ménière's syndrome, all medical treatment is more or less empiric. The success of any one form of treatment varies greatly with different physicians. Because of the spontaneous variations in the frequency of attacks, it is not easy to evaluate the results that are obtained Success with medical treatment is usually limited to relief of the attacks of vertigo. The tinnitus, which in some patients is a very annoying symptom, is usually not benefited by therapy. The only treatment which assures permanent relief from the recurrent attacks of vertigo is surgical destruction of the labyrinth or section of the vestibular branch of the eighth nerve on the affected side This will prevent the occurrence of subsequent attacks of vertigo but will not, in the majority of patients, abolish the tinnitus Destruction of the labyrinth or surgical section of the nerve are major surgical procedures; since good results may be obtained in many cases by medical treatment, it is wise to try the latter before resorting to surgery.

Our experience in the medical treatment of Ménière's syndrome has been limited to the use of the salt-free diet and potassium chloride therapy.

Potassium Chloride Therapy. The treatment, which is easy to administer, consists of the oral administration of 8 Gm. of potassium

percentage of patients. Horton (14) and co-workers found that dihydroergotamine methyl sulfonate was just as effective as ergotamine tartrate in relieving the symptoms in migraine, and that toxic symptoms were three times as frequent with ergotamine tartrate as with the dihydroergotamine.

Ergotamine compounds, when effective, relieve only the attack and have no influence on the frequency of the attacks, even when given regularly in small doses between the attacks. For this reason, strenuous efforts must be directed toward the elimination of factors which play a role in the production of the attacks

Other Forms of Therapy. Other forms of medical therapy, which have not been used in enough patients to evaluate their results, are inhalation of pure oxygen, intravenous injection of vitamin B, and histamine desensitization

Various attempts have been made to prevent the occurrence of migraine attacks by surgical procedures. The only ones that have proved of any value are based on the hypothesis that headaches are due to dilatation of the cranial extracerebral vessels. Good results have been reported in a small number of cases from ligation and sectioning of the middle meningeal or temporal arteries. There have not been a sufficient number of such cases, however, to evaluate this mode of therapy.

MÉNIÈRE'S SYNDROME

Ménière's syndrome is characterized by recurrent attacks of severe vertigo, with nausea and vomiting. The attacks have an abrupt onset, sometimes with such violence that the patient is thrown headlong to the ground During the attack there is severe vertigo accompanied by nausea, vomiting, pallor, and excessive sweating The symptoms in a single attack may last from a few hours to a day or more The frequency of attacks may vary from several a week to one in several years. Tinnitus, in one or both ears, is frequent during the attacks, and may also be present in the interval between them Impairment of hearing of the nerve type is often present, increasing in severity during an attack. Tests of labyrinthine function may show a slight disturbance of labyrinthine activity.

The pathologic physiology of the attacks is not known. Sections of the vestibular nerve do not show any significant pathology. It

accompany laughing or crying are not improved in all patients in whom these symptoms are present.

Amphetamine Sulfate Therapy The dosage of amphetamine sulfate required to relieve the symptoms varies from $^1/_6$ grain (10 mg) to 2.5 grains (170 mg) daily. The optimum amount is determined by starting with a dose of $^1/_6$ grain, given on arising in the morning, and increasing this dosage by increments of $^1/_6$ grain spread through the day until the attacks of diurnal sleep stop. Approximately $^1/_2$ to 1 grain (30–60 mg.), in doses of $^1/_6$ to $^1/_3$ grain (10–20 mg.) 3 times daily, is the amount required in the average case The timing of the dosage should be such that the patient receives sufficient medicine to keep him awake during the day, and allows for normal sleep at night. The last dose of the day is usually given between 3.00 and 6 00 P.M, unless the hour of retirement is to be unusually late.

Untoward effects of this drug in narcoleptics are uncommon, and are usually of minor significance Overdosage, or improper distribution of the dosage, is followed by a disturbance (prevention) of nocturnal sleep, restlessness, excitability, or nervousness. These symptoms are alleviated by reducing the dosage or changing the time of ingestion. Constant use of the drug over a period of years produces no untoward effects on the cardiovascular, renal, or blood-forming organs, nor does tolerance to its therapeutic effect develop in the patient.

Ephedrine Therapy. While ephedrine is usually not as effective as benzedrine sulfate in treating the symptoms of narcolepsy, satisfactory results can be obtained in many cases As with benzedrine, it is necessary to determine in each patient the optimum size and distribution of the dose In the usual case, ⅜ to ¾ grain (25–50 mg) of the sulfate, 2 to 4 times daily, is the range of dosage. Such doses are well tolerated and there are no serious side effects

FAMILIAL PERIODIC PARALYSIS

Familial periodic paralysis is a rare disease, characterized by periodic transient attacks of paralysis of the muscles of the trunk and extremities The musculature of the face, throat, palate, and tongue are usually spared, as are also the muscles of respiration During an attack, the reflexes are absent and the muscles cannot

chloride daily, given in the form of a 25 per cent aqueous solution, 2 teaspoonfuls 4 times a day The only untoward side effect of this compound is gastric distress, which can be prevented by giving the potassium chloride in enteric-coated tablets Potassium compounds should not be given to patients with kidney diseases associated with nitrogen retention in the blood.

Salt-Free Diet and Ammonium Chloride Therapy. Decrease in the frequency of attacks has been reported as result of administering ammonium chloride and a salt-free diet The difficulties in preparing such a diet, however, make this form of treatment of less practical value than the potassium chloride treatment.

NARCOLEPSY

Narcolepsy is a symptom complex characterized by periodic attacks of an uncontrollable desire to sleep, sudden loss of postural tonus (cataplexy), and pathologic weakness of the musculature during emotional reactions, such as laughing (*lachslag*) or crying The periodic attacks of somnolence which are the cardinal and often the only symptom of the syndrome, differ from normal sleepiness in their frequency and in their occurrence at times inconsistent with normal. When the desire for sleep develops, the narcoleptic is apparently unable to resist it, he is forced to lie or sit down and falls into an apparently normal slumber from which he can readily be awakened, or, if undisturbed, will awaken after an interval varying from a few minutes to an hour or more. Narcoleptics require from several to many such naps each day, in addition to a full night's sleep. The etiology of the disturbance is unknown, and there is no anatomic pathology It may follow infections of, or trauma to, the nervous system, but in the majority of cases there is no history of an antecedent injury to the nervous system

The treatment of narcolepsy is an empiric one, directed toward the prevention of the attacks of somnolence. Janota in 1931 and Doyle and Daniels in the same year reported that ephedrine prevented these attacks Prinzmetal and Bloomberg (18) in 1935 demonstrated that amphetamine sulfate (benzedrine sulfate) was more effective than ephedrine, and it is now the standard treatment The somnolence can be relieved in practically all cases, but the cataplexy and the attacks of generalized muscular weakness which

arising in the morning than they are as the day progresses, and similarly, when eating, the first few mouthfuls are swallowed much more readily than later ones Rarely, the symptoms are so severe that the patient is bedridden and requires tube feeding. The disease may occur at any age. The etiology is as yet unknown. It has been known to develop following acute infections of the nervous system, and in the course of, or following, exophthalmic goiter. Pregnancy usually produces a beneficial effect on the disease, especially in the later months; occasionally, however, myasthenia gravis may have its onset during pregnancy. The anatomic pathology of the disease is unsatisfactory. Lymphocytic infiltration of the muscles and adenomatous enlargement of the thymus have been reported in some cases. In recent years, however, some understanding of the pathologic physiology of the disease has been obtained. According to the advocates of the theory of the chemical transmission of the nerve impulse, acetylcholine is the intermediary in the transmission of the nerve impulse at the myoneural junction. Stimulation of a nerve releases acetylcholine, which transmits the impulse to the muscle. Prolonged action of the liberated acetylcholine is prevented by the presence of esterases which destroy it According to this theory, in patients with myasthenia gravis there is a deficiency in the formation of acetylcholine or an excess of the esterases which rapidly destroy the acetylcholine, thus preventing its normal action Action of the esterases is inhibited by physostigmine compounds and allows the action of the acetylcholine to be prolonged

The treatment of myasthenia gravis with physostigmine was discovered empirically by Walker in 1934. The effectiveness of neostigmine, a physostigminelike compound, in relieving the fatigability and increasing the strength of the muscles of patients with myasthenia gravis is so striking and constant that Viets and associates (21a,b) have used it as a diagnostic aid.

In the diagnostic tests, 3 cc of a 1:2,000 solution (1.5 mg.) of neostigmine plus 0 01 grain (0 66 mg.) atropine sulfate are given intramuscularly. Objective and subjective improvement in muscular power is recorded at intervals from 10 minutes to 8 hours following the injection, and graded from 0 to 4. The results in a case of myasthenia gravis taken from the reports of Viets and Mitchell (21a) are shown in Table I. The average score of patients with myasthenia gravis, according to this method, is about 30, whereas

be stimulated electrically. The duration of an attack varies from a few hours to 24 or more, and the frequency from many attacks a month to one or two a year. As its name implies, there is a familial occurrence of the disease, but there is no known etiology or microscopic pathology. It has been shown that at the onset of an attack the potassium content of the serum drops acutely—from a normal level of 18 to 23 mg. per hundred cubic centimeters to 10 mg or less. There is also a slight lowering of the carbon dioxide combining power of the serum. The weakness disappears when the serum potassium returns to normal levels (19). Attacks are prone to follow ingestion of a large carbohydrate meal.

Since the cause of the disturbed potassium metabolism is unknown, treatment is directed toward restoring the potassium level of the serum to normal (20). Immediately upon onset of an attack, potassium chloride should be given by mouth, in doses of 5 to 15 Gm, according to the amount required for relief of the paralysis. This can be given in a 25 per cent aqueous solution, 2 Gm (8 cc.) every hour, until the patient has recovered. In extremely severe attacks with weakness of the muscles of respiration and deglutition, artificial respiration should be applied and potassium chloride administered intravenously in doses of 50 cc. (1 Gm.) of a 2 per cent solution.

Potassium chloride should be taken daily, in order to prevent, if possible, the occurrence of the attacks of paralysis The amount required varies with each patient, and must be determined by trial. As a rule, from 4 to 8 cc. of the 25 per cent solution (1 to 2 Gm) of potassium chloride is administered 2 to 4 times daily. Such doses can be tolerated without any untoward effects Heavy carbohydrate meals should be avoided.

Myasthenia Gravis

Myasthenia gravis is a disease in which there is pathologic fatigability of normal-appearing muscles Any or all of the muscles of the body may be affected, but the bulbar (eye, facial, palatal, and pharyngeal) musculature is most frequently involved. A characteristic of the disease is that the first few contractions of a muscle are fairly normal, but succeeding contractions become progressively feeble The patients are therefore usually much stronger on

of hyperplasia of the gland. A tumor was present in the thymus in only 2 of Blalock's 20 patients and in 4 of Viets' 15 patients. It is difficult to evaluate the results obtained by removal of the thymus gland, since spontaneous remissions in the course of the disease have been known to occur. The operative mortality rate is about 20 to 25 per cent. Removal of the gland is therefore not recommended for patients whose symptoms are well controlled by a moderate amount of neostigmine or for those over 50 years of age Only a few of the patients whose thymus glands have been removed have been able to get along without the use of neostigmine; in the vast majority, improvement consisted in an ability to perform their daily tasks on a smaller dose of neostigmine.

Dosage and Administration of Neostigmine. Neostigmine can be given intravenously, intramuscularly, and by mouth. For obvious reasons, oral administration is the preferred one Intramuscular injection is useful as a diagnostic test and as a supplement to the oral route in patients with very severe symptoms Intravenous administration is rarely necessary, and should be used only in extremely severe cases The amount of neostigmine administered should be sufficient to control the symptoms and must be individually determined

Parenteral Route. From 1 to 3 cc of a 1 2,000 solution (0 5–1 5 mg) of neostigmine, plus 0 01 grain (0 66 mg) atropine sulfate may be injected intramuscularly, 2 or 3 times daily, or more often if needed, to control the symptoms

Oral Route Approximately 60 mg (1 grain) of neostigmine, given by mouth is as effective as 1 mg (0 015 grain) given intramuscularly The response to oral administration is slower and is less complete The tablets for oral administration are each of 15 mg. (0 225 grain) The maintenance dose varies from 1 to 2 tablets in 24 hours to 1 to 2 tablets every hour during the day. The first dose in the morning should be given before arising and in severe cases it is advisable to have on hand an ampule for muscular injection

To prevent or alleviate the visceral disturbances which occur in some patients, such as salivation, abdominal discomfort, cramps, diarrhea, or desire to defecate, 3 to 15 drops of tincture of belladonna, or 0 005 grain (0 3 mg) of atropine sulfate, may be taken with the prostigmine Potassium chloride, ephedrine, or benzedrine sulfate may be useful along with the prostigmine Potassium chloride

the score of patients with muscular weakness due to other diseases of the nervous and muscular systems, such as muscular atrophies, dystrophies, and the like, is less than 10. Patients with asthenia due to psychic causes may show a high score in the subjective column, but such cases can be readily detected by the absence of any objective improvement. As may be seen from Table I, the effects of

TABLE I
Results of Injection of Neostigmine and Atropine Sulfate in a Patient with Myasthenia Gravis (21a)

Time after injection	Improvement in Muscular Power	
	Objective score	Subjective score
10 minutes	3	2
30 minutes	4	4
1 hour	4	4
2 hours	3	4
4 hours	2	2
6 hours	0	1
8 hours	0	0
Totals	*16* +	*17* = 33

neostigmine are transient and last for only a few hours; it is claimed by some that the weakness following this period of improvement is greater than that present before injection of the drug. Whether this is true or not, the use of neostigmine enables patients with myasthenia gravis to live more normal lives than was possible with any other treatment heretofore used Potassium chloride and ephedrine sulfate have been found to be useful as adjuvant therapy with prostigmine.

Guanidine hydrochloride has also been found effective in relieving the myasthenic symptoms It can be used alone or in conjunction with neostigmine According to Viets (22), it is much less effective than neostigmine, but it is of definite value in some cases when used in conjunction with neostigmine since the dose of the latter drug can be decreased

The role of the thymus gland as a cause of myasthenia gravis is as yet unclear Beneficial effects from the removal of the thymus gland have been reported by Blalock (23) and by Viets (22). Favorable results, when they occurred, were not related to the presence

such as Parkinsonism, dystonia, or athetosis, and the spasticity of muscles which is present in patients with lesions of the corticospinal tracts. Relaxation of the muscles can be obtained with single injections of curare (24) which are sufficient to produce paralysis of the voluntary muscles, and some degree of relaxation may persist for 24 to 48 hours after complete recovery of voluntary power. Preliminary experiments with β-erythroidine (25) given orally have not yielded any significant results. This treatment has not, however, been tried on a sufficiently large number of patients for a long enough period to determine the optimum dosage or whether any significant degree of relaxation of spastic or rigid muscles can be obtained with dosages which do not produce muscular weakness. The use of these compounds is not without serious dangers, and at the present time should be limited to experimental clinics where proper precautions are observed.

Surgical Treatment of Muscular Rigidity, Spasticity, and Abnormal Movements. The disappointing lack of success of medical treatment of Parkinsonism, athetosis, dystonia, chorea, or spastic paralysis led to the consideration of surgical methods of relieving these conditions. Operations have been performed on various levels of the central nervous system A partial list of the operative procedures includes: (*1*) section of the anterolateral tracts in the spinal cord, (*2*) section of the lateral tracts (including the corticospinal) in the cord, (*3*) removal of portions of the cerebellum, (*4*) extirpation of the head of the caudate nucleus, (*5*) section of the ansa lenticularis, and (*6*) removal of varying portions of the cerebral cortex (26)

Varying degrees of success have been reported by the neurosurgeons who have tried these operations These results have not appeared very impressive to unbiased observers, and the multiplicity of the operative procedures would seem to indicate that the solution of the problem of abnormal movements and abnormal muscular tension has not as yet been found in surgical procedures.

PARKINSONISM (PARALYSIS AGITANS)

Diffuse damage to the basal ganglia by infections, toxic agents, arteriosclerosis, or senile changes may cause the syndrome known as Parkinson's disease, or paralysis agitans. The symptoms are

may be given in doses of 1 to 2 Gm. 3 or 4 times daily, and ephedrine in doses of ⅜ grain (25 mg.) at similar intervals.

Guanidine hydrochloride can be used alone or in conjunction with prostigmine. It is given in the form of 125 mg. tablets by mouth The dosage should be regulated according to the effect on the symptoms and the development of untoward side reactions. Guanidine is more likely to produce visceral disturbances, than is prostigmine, and its administration is also accompanied by troublesome paresthesias around the mouth and the finger tips.

Diseases of the Nervous System with Abnormal Movements or Increased Muscle Tone

Diseases which affect the basal ganglia cause abnormal movements and disturbances of muscle tone. These abnormal movements are described by such terms as choreic, athetotic, hemiballistic, or dystonic, but by far the most common is the alternating tremor which is present in the Parkinsonian syndrome. In some patients, abnormal movements may be accompanied by a disorder of muscle tone, while in others a muscular rigidity is the main sign of their disease.

Diseases of the cerebellum or its connections with the brain stem may also cause abnormal movements The tremor in these patients differs from that seen in patients with basal ganglia diseases, appearing only when the patient attempts to use the muscles voluntarily Lesions of the corticospinal tract at any site from the cerebral cortex to the end of the spinal cord are usually accompanied by an increase in muscle tone which is described by the term "spasticity", it differs from the increased muscle tone seen in patients with basal ganglia disease in that resistance to passive movement is greatest at the start of the movement and frequently relaxes suddenly with continuation of the forceful passive movement (clasp-knife like).

The medical treatment of these disorders has been unsatisfactory except in Parkinsonism, which will be considered in detail later

Treatment of Muscular Spasticity and Rigidity with Curare and Curarelike Compounds Curare and curarelike compounds, especially β-erythroidine, have been given in an attempt to relieve the rigidity of the muscles which occurs with basal ganglia diseases,

valuable as an adjuvant to combat the excessive drowsiness or the mental depression which is common in these patients Sedatives such as phenobarbital are contraindicated, since they tend to increase these symptoms, and occasionally the rigidity and tremors as well

The alkaloids of belladonna root, singly or in combination, have proved to be the most satisfactory mode of therapy. The preparation to be used is of slight importance, in comparison to the regulation of the dosage. Optimum results are usually obtained with a dosage which approaches the maximum tolerated dose. As a general principle, treatment should be started with very small amounts of the alkaloid, gradually increasing it over a period of weeks until the development of untoward symptoms indicates that further increases are inadvisable. The optimum dose is usually slightly below this level The side effects of overdosage of drugs of the belladonna group are, in the order of frequency of their occurrence, dryness of the mouth, pupillary dilatation and impairment of the ability to converge, urinary retention, dysphagia, drowsiness, gastrointestinal symptoms (nausea, vomiting, abdominal cramps, diarrhea), diplopia, headache, and mental confusion Most of these symptoms can be avoided by starting with very small doses spaced at intervals throughout the 24 hours, and increasing the dose slowly Occasionally, it is advisable to continue with a dose that produces mild reactions, such as dryness of the mouth and difficulty in focusing the eyes. The dryness of the mouth can be combated by giving the drug after meals, or having the patient keep a small piece of hard candy, such as a lemon drop, in the mouth or by chewing gum. Difficulty in convergence may be lessened by instilling 1 or 2 drops of 0 5 to 1 per cent aqueous solution of eserine, but since the prolonged use of eserine tends to produce conjunctivitis, eye glasses with a 1 to 2 diopter plus lens can be prescribed for reading Exposure to excessive heat should be avoided because perspiration is inhibited Injections of pilocarpine in doses of $^1/_{10}$ grain can be used to combat the side effects of belladonna

The alkaloids of belladonna in common use and their dosage are as follows·

Scopolamine or hyoscine is usually given in the hydrobromide form, starting with a daily dose of $^1/_{200}$ grain (0 33 mg) and increasing after 3 to 5 days to $^1/_{200}$ grain (0 33 mg) twice daily, and

mainly due to a disturbance of muscular activity resulting from excessive rigidity or tremor The rigidity of the muscles causes a slowness and poverty of motion, a masklike facies, dysarthria, and a stooped posture. When the muscles are passively flexed or extended, the movement is not a smooth and regular one but an interrupted one (cogwheel phenomenon). The tremor is of alternating type, at the rate of several times per second, and commonly involves the extremities of one or both sides and occasionally the muscles of the jaw and neck One characteristic of this tremor is that it is greatest when the muscles are at rest, and tends to disappear or decrease in amplitude when voluntary movements are attempted. Other symptoms may be present in addition to the rigidity and tremor, such as excessive salivation, impaired eye movements, particularly convergence, oily skin, nocturnal incontinence, pains in the extremities, respiratory tics, and spasmodic contractions of the eye muscles. The last mentioned is known as oculogyric crisis, and is found almost exclusively in the parkinsonism secondary to encephalitis lethargica

Signs of parkinsonism may develop in a patient at the time of the acute involvement of the nervous system by encephalitis lethargica, or they may not appear until several or many years later. The symptoms may, in rare cases, develop in children without any antecedent infection (juvenile paralysis agitans) and in young or middle-aged adults without arteriosclerosis. They may also follow injury to the basal ganglia by arteriosclerosis, carbon monoxide poisoning, electric shock, syphilis, multiple sclerosis, cerebral trauma, and tumors of the brain

The treament of parkinsonism is entirely symptomatic. Complete relief from the symptoms is rarely, if ever, possible, but a moderate degree of improvement can be obtained in practically all patients, and occasionally the results of treatment are extremely gratifying. The best results are obtained in patients with a predominance of spasticity and little or no tremor With treatment, these patients say they feel looser, that they can get around better, and perform their routine tasks more easily The salivation, pain in the extremities, and the retropulsion are also usually amenable to therapy, but the tremor is often quite resistant Oculogyric crises, which respond poorly to the belladonna alkaloids alone, may be relieved by combining them with amphetamine sulfate This latter drug is especially

to facilitate division One tablet is given as the first dose, and increases in dosage are the same as outlined above for vinobel. The usual range of dosage tolerated is, 1 to 3 tablets, 3 or 4 times daily.

Amphetamine sulfate (benzedrine sulfate) can be used in conjunction with any of the belladonna alkaloids. It is particularly indicated when the patients complain of excessive somnolence or mental depression, or when oculogyric crises are present Dosage should be regulated according to tolerance and therapeutic effect. From 10 to 20 mg, once or twice daily in the forenoon, is usually sufficient.

Vascular Lesions of the Nervous System

Vascular lesions of the nervous system can be divided into four groups: (*1*) cerebral hemorrhage, (*2*) cerebral thrombosis, (*3*) cerebral embolism, and (*4*) primary subarachnoid hemorrhage Traumatic lesions of the brain producing subdural, extradural, or intracerebral hemorrhages or secondary hemorrhages into the subarachnoid space are discussed elsewhere

The diagnosis of a vascular lesion in the brain can usually be made without difficulty, but the differential diagnosis between the various types of vascular lesions is not so simple The sudden onset, with or without loss of consciousness, of focal cerebral symptoms such as hemiplegia, aphasia, or hemianopia, is with a few exceptions diagnostic of a cerebrovascular lesion

CEREBRAL HEMORRHAGE, CEREBRAL THROMBOSIS

Cerebral hemorrhage and cerebral thrombosis constitute by far the major part of the lesions of the central nervous system due to vascular disease. These lesions may occur at any age but, since they are usually a manifestation of cerebral arteriosclerosis, they occur predominantly in middle and late life. The differential diagnosis between cerebral hemorrhage and cerebral thrombosis can often be made if the following points are kept in mind: (*1*) Extravasation of the blood into the substance of the brain causes an acute rise in intracranial pressure and results in headache, nausea, vomiting, and occasionally convulsions Such symptoms are rare in patients with a cerebral thrombosis (*2*) The presence of signs of meningeal irritation, such as stiffness of the neck and Kernig's sign,

after an equal interval of time to $^1/_{200}$ grain 3 times a day. Further increases should be made by increments of $^1/_{200}$ grain until the optimum dose is obtained In rare cases dosages as high as $^1/_{50}$ grain 3 to 4 times daily may be tolerated.

Stramonium, which contains a mixture of the alkaloids atropine and hyoscyamine, can be administered as the tincture or in the form of pills. Treatment is started by giving a 2 $^1/_2$ grain pill (166 mg.) once daily, increasing after 3 to 5 days to a pill twice daily, with further increases by increments of 2 $^1/_2$ grains until the optimum dose is reached From 10 to 15 grains (0 66–1.0 Gm.) daily, divided into 4 or 5 doses, can be tolerated by many patients

Tincture of belladonna can be given, but it usually produces untoward symptoms before the optimum therapeutic dose is reached. It can be tried by giving 5 minims once daily and increasing this to 5 minims twice daily and then to 3 times daily. Further increases to the limit of tolerance should be by increments of 5 minims

Bulgarian belladonna has recently received considerable attention because of the therapeutic effect of its root administered as a tincture, or in white wine. The favorable results with this form of treatment are not due to any special quality of the Bulgarian root, but are related to the care which is taken in regulating the dosage. In addition, it is probable that a combination of the various alkaloids of belladonna root is more effective in nontoxic dosages than any single one of its various alkaloids. There are two preparations in common use which contain a mixture of the belladonna alkaloids. The first is a desiccated white wine extract of U S P belladonna, which is marketed in tablets containing 0 4 mg and 0 8 mg. of total alkaloid under the name of vinobel. The method of administration is to start with 1 tablet of 0 4 mg and gradually to increase the dose at intervals of 3 to 5 days until the maximum therapeutic effect is obtained, or until further increase in dosage is contraindicated by the appearance of untoward side symptoms. Most patients will be able to tolerate 3 to 6 mg in a period of 24 hours and some patients, especially the younger ones with postencephalitic parkinsonism, will tolerate much larger doses

The second preparation is a synthetic compound containing the various alkaloids in the same proportion as they occur in the Bulgarian root. It is manufactured in tablets, containing 0 5 mg of total alkaloid, under the name of rabellon The tablets are scored

high cerebrospinal fluid pressure as measured by lumbar puncture, craniotomy with removal of the clot will increase the possibilities of recovery

Cerebral thrombosis is in most instances due to arteriosclerosis of the vessels, but occasionally syphilis may be the etiologic factor. For this reason, serologic tests should be performed on the blood and cerebrospinal fluid; if syphilis is found to be present, antisyphilitic treatment should be given. It should be emphasized, however, that such treatment will have no appreciable effect on the pre-existing cerebral lesion and is given mainly to prevent the occurrence of subsequent vascular occlusions.

Treatment after the patient has recovered from the shock is directed toward relief or amelioration of the neurologic defect produced by the vascular lesion Splints should be applied to prevent stretching of paralyzed muscles while the limbs are at rest Usually, anterior splints to the forearm and posterior splints to the ankle joints, are sufficient Physiotherapy should be started as soon as the patient is strong enough for the treatments The paralyzed muscles should be massaged several times daily and all joints of the paralyzed limb passively moved to prevent the development of contractures. With return of muscle power, the patient should be allowed to resist the passive movements The patient should be allowed to sit up in a chair as soon as possible, to walk with support on both sides, and finally to walk with a crutch or a cane The return of dexterity in the hands can be aided by giving the patient a rubber ball to squeeze As movement returns, more intricate use of the hand muscles should be encouraged, such as writing or drawing. The results obtained depend in a large part upon the diligence and persistence of the therapist No degree of paralysis should be considered hopeless, and treatment should be continued for at least a year or two before abandoning hope of further improvement

Speech disorders such as dysarthria and aphasia can be improved to a remarkable degree by persistent effort Whenever possible, a psychologist who has had experience in retraining patients with speech defects should give the patient daily exercises. If no such therapist is available, the physician himself can accomplish a great deal Constant, persistent efforts are required, starting with simple pictures and sound and gradually increasing the complexity of the symbols as the speech functions return Training should begin as

indicate the rupture of a blood vessel with extravasation of blood into the ventricular or subarachnoid space. (3) Signs of widespread damage to the brain are much more common in patients with cerebral hemorrhage. Conjugate deviation of the head and eyes and a bilateral Babinski reflex are in favor of this diagnosis. (5) A bloody cerebrospinal fluid under increased pressure is of course diagnostic of hemorrhage.

The differential diagnosis between cerebral hemorrhage and thrombosis is important because of the vastly different prognosis and treatment Cerebral hemorrhage of any appreciable size is almost always fatal, whereas the chances for recovery from cerebral thrombosis are good. The main difference in the treatment of these two conditions is that surgical removal of the clot is at times indicated in patients with a cerebral hemorrhage

The treatment of patients with cerebral thrombosis and cerebral hemorrhage is divided into two stages. In the acute stage, treatment is directed toward saving the life of the patient After recovery from the shock, therapy is directed toward obtaining the maximum return of function of paralyzed limbs

Nursing care is of prime importance in the acute stage of the condition If the patient is comatose, he should be given fluid and glucose either by vein or by nasal catheter If the patient is conscious, a liquid and soft solid diet can be given by mouth Venisection is of doubtful value, and should be used only when signs of congestive heart failure are present The bladder should be emptied as often as necessary by catheterization The patient should be shifted in bed frequently, to prevent the development of hypostatic pneumonia and bed sores The bed clothes should be changed immediately when they are soiled Lumbar puncture is of value in the differential diagnosis, but it is of doubtful aid in therapy. When a large amount of blood has been extravasated into the subarachnoid space, lumbar puncture may help to relieve the increased intracranial pressure; in such cases it can be repeated after 12 to 24 hours

Since cerebral hemorrhage is fatal in most cases, the possibility of the surgical removal of the clot should be considered. In many patients there is such a degree of shock that the patient will not be able to tolerate an operation But if the patient survives the initial shock and continues to have an increased intracranial pressure, as manifested by the development of choked disk, or by a

aneurysm, there is a sudden sharp pain in the head, usually in the occipital region, followed by a severe headache accompanied by nausea and vomiting. Mental cloudiness or stupor may follow in a few hours or days. Convulsions are rare, and the development of a hemiplegia or other focal neurologic sign usually indicates that the hemorrhage is primarily into the cerebrum Focal neurologic signs are occasionally present in primary subarachnoid hemorrhage, since the aneurysm may be so located as to rupture both into the cerebrum and into the subarachnoid space.

Treatment of primary subarachnoid hemorrhage is directed toward relief of the increased intracerebral pressure and of the signs of meningeal irritation. Both of these respond very readily to the removal of cerebrospinal fluid by lumbar puncture There is no evidence that removal of fluid tends to increase the bleeding, and the dramatic clinical improvement following lumbar puncture makes this form of treatment almost imperative. Sufficient fluid should be removed at each puncture to bring the pressure to a low normal level. The frequency of the puncture should be determined by the rapidity of the recurrence of symptoms and the level of intracranial pressure encountered at the previous puncture Usually two or more punctures are required for the first several days, single punctures for the next 3 to 6 days, and occasional ones during the next 2 weeks.

The patient should be kept in bed until all the symptoms have disappeared and until the cerebrospinal fluid is entirely normal. Because of the danger of subsequent rupture of the aneurysm, the period of convalescence should be long The patient should be kept in bed for at least 3 to 6 weeks after the symptoms have subsided, and the return to normal activity should be very gradual Heavy lifting and all forms of physical strains should be avoided. Bowel movements should be kept loose by the use of mineral oil. The role of surgery in the treatment of aneurysms is still under debate. It is the feeling of some observers (27) that the likelihood of a recurrence of the rupture is so high that surgery is indicated in the vast majority of these patients. If, on cerebral arteriography, an aneurysm is visualized, they advise that craniotomy should be performed and the aneurysm ligated or coagulated In deciding for or against operation, the high mortality rate of this operation should be considered against the possibility of a repeated rupture of the aneurysm.

soon after onset of the speech disturbance as possible, but good results can be obtained even with patients who have been completely aphasic for many weeks or months before treatment is started.

CEREBRAL EMBOLISM

Occlusion of a cerebral vessel by embolism leads to necrosis of the brain tissue supplied by this vessel, in a manner exactly like that which occurs after thrombosis of the vessel. The most common causes of cerebral embolism are diseases of the heart and septic processes in the lung. The signs and symptoms of cerebral embolism are the same as those of cerebral thrombosis, and the diagnosis of embolus is made when signs of a cerebral vascular lesion occur in a patient with a focus from which an embolus could dislodge. Examination of the heart and lungs for a possible focus, culture of the blood for bacteria, and examination of the cerebrospinal fluid are of course important aids in the diagnosis.

Treatment of the patient in the acute stage and treatment of the neurologic defects produced by cerebral embolism are in no wise different from the treatment of defects produced by cerebral thrombosis. The prognosis for life and complete recovery is, of course, not as good in patients with an embolism, because of the gravity of the underlying pathologic process in the heart or lung

PRIMARY SUBARACHNOID HEMORRHAGE

Blood may be extravasated into the subarachnoid space as a result of contusions and lacerations of the brain by trauma, or by rupture of an intracerebral hemorrhage into the ventricles or subarachnoid space. The presence of blood in the subarachnoid spaces in these cases is secondary to the brain lesion. Primary subarachnoid hemorrhage is due to the spontaneous rupture of an aneurysm in the subarachnoid spaces These aneurysms are usually considered to be the result of a congenital defect in the vascular wall. Occasionally, the aneurysms may be of arteriosclerotic or mycotic and, rarely, of syphilitic origin. The circle of Willis is the most common site for aneurysms. Occasionally, because of its size, an aneurysm may press on one or more cranial nerves and cause pain in the head or paralysis of motor nerves, but as a rule aneurysms produce no symptoms until they rupture. With rupture of the

When the patient has recovered from surgical shock or if this complicating factor is not present, the general treatment consists of administration of the proper amount of fluids and nutrition by mouth or parenterally, care of the skin, prevention of bladder distension, and evacuation of the bowels. The eyes should be protected from drying Blood or cerebrospinal fluid should be wiped from the external ear, but under no circumstances should the ear be irrigated or probed Drainage of cerebrospinal fluid from the nose should not be treated locally but must be stopped by an intracranial operation. Drugs should be used only as indicated The usual analgesic drugs can be given for headache, but morphine should be avoided If the patient is unduly restless, he may be quieted by small doses of the barbiturates or by rectal administration of paraldehyde in doses of 4 to 6 drams in 4 ounces of starch solution Caffeine, 7$^1/_2$ grains intravenously, can be given as a stimulant if needed.

In addition to the general treatment, special measures may be necessary to reduce increased intracranial pressure There is some disagreement regarding the best method of controlling intracranial pressure. An increased pressure can be reduced by removal of cerebrospinal fluid by lumbar puncture or by dehydrating the patient. Dehydration can be accomplished either by the use of hypertonic solutions—50 per cent glucose in doses of 100 cc, intravenously, or 4 ounces of a saturated solution of magnesium sulfate by rectum—or by a drastic limitation of fluid intake These measures should be used only as a temporary expedient, because of the danger of excessive dehydration. In our opinion, it is wiser to give the patient an adequate fluid intake and control the intracranial pressure by removal of cerebrospinal fluid by lumbar puncture Lumbar puncture should be performed as soon as the patient has recovered from shock to aid in the diagnosis of the type of brain injury The intracranial pressure should be accurately measured and the fluid examined in the laboratory The puncture should be repeated at intervals of 8 to 24 hours until the pressure returns to normal and the blood has been removed

Removal of fluid by lumbar puncture is often the only measure needed to control the intracranial pressure, and it will allow administration of the proper amount of fluids to prevent toxic dehydration Only in very rare cases is decompression by craniotomy necessary

Trauma to the Nervous System

Traumatic injuries to the central nervous system are usually considered to be the province of the surgeon. This is certainly true for the cases with penetrating wounds of the skull, compound or depressed fractures of the skull, and cases with subdural or extradural hemorrhage The majority of the patients with head injuries, however, have no injury to the skull except possibly a linear fracture. This group, the so-called closed head injuries, do not need any operative treatment and are often cared for by the neurologist or internist. *It is essential, however, that the physician in charge of these patients be constantly on the alert for the development of symptoms or signs which indicate the need for operative intervention.*

The patients with closed head injuries can be divided into three subgroups, according to the severity of the brain injury: (*1*) concussion, (*2*) edema and congestion, and (*3*) contusion and laceration The term concussion implies that there has been a minor injury to the brain which caused a momentary loss of consciousness without any objective signs of cerebral trauma, and without any abnormalities in the cerebrospinal fluid The diagnosis of edema and congestion presupposes a more serious injury to the brain, with an increase in the intracranial pressure resulting from edema and interference with the circulation The terms cerebral contusion and laceration are used when the brain has been bruised or torn, with resulting hemorrhage into the subarachnoid space or substance of the brain The cerebrospinal fluid in such cases is bloody and under increased pressure. Focal neurologic signs may or may not be present The following *are complications of skull or brain injury which require* special treatment extradural and subdural hematomas, meningitis, brain abscess, osteomyelitis or infected wounds of the scalp, arteriovenous aneurysm, cerebrospinal rhinorrhea, and aerocele

Most important in the treatment of patients with head injuries is the general care in the immediate period following injury Surgical shock should be treated by placing the patient in a warm bed with the feet elevated, and by the transfusion of 250 cc. of whole blood. Diagnostic procedures, such as lumbar puncture and roentgenograms of the skull, and special surgical treatment of the injury to the scalp or skull, should be deferred until the patient recovers from shock

longer for the most severe injuries. Specific directions should be given as to general hygiene and the avoidance of overmedication Alcohol should be strictly avoided

COMPLICATIONS OF HEAD INJURY REQUIRING SURGICAL TREATMENT

Extradural Hemorrhage The diagnosis of extradural hemorrhage is usually not difficult when the classic sequence of events occurs, i e, an injury to the head accompanied by coma followed by a recovery of consciousness and a subsequent lapse into coma coincidental with the development of a hemiplegia The diagnosis is more firmly established if roentgenograms of the skull show a fracture line running through the groove of the middle meningeal artery or one of the larger cerebral venous sinuses It must be remembered that this classic picture is only present in a little more than half of the cases. For example, the brain may be so injured as to prevent the return of consciousness before the symptoms of the extradural hemorrhage are superimposed. Extradural hemorrhage is treated by immediate operation, with evacuation of the clot and ligation of the bleeding vessel A complete cure can be obtained if operation is performed speedily. Undue delay will almost invariably result in death.

Subdural Hematoma. Signs and symptoms of subdural hematoma may develop in the period immediately following a head injury or they may be delayed for several months. The diagnosis of an acute subdural hematoma is often extremely difficult It should be suspected in all patients who do not respond to routine treatment, especially if focal neurologic symptoms (hemiplegia, for example) develop or if there are alternating periods of stupor and consciousness A shift of the pineal gland on the roentgenograms of the skull and changes in the electroencephalogram may help in the diagnosis If the presence of a subdural hematoma is suspected, small trephine holes should be made in the temporal region on both sides. When a hematoma is present, it can be evacuated by suction

The symptoms of chronic subdural hematoma are those of an expanding intracranial lesion This diagnosis should be entertained whenever such symptoms (headache, focal neurologic signs, coma, choked disk) develop within a few weeks or months after a head

for relief of intracranial pressure from cerebral edema. Dehydration or removal of fluid by lumbar puncture is of no value in the therapy of extradural or subdural hemorrhages.

Roentgenograms of the skull should be taken at some time before *the patient's discharge from the hospital. In the vast majority of* cases, nothing is gained by rushing the patient to the roentgenologic department immediately on admission. On the contrary, valuable time is lost in the institution of rational therapy. The presence or absence of a linear fracture of the skull is of no practical significance in the early management of the case. Roentgenograms of the skull are of definite value in the diagnosis of extradural hemorrhages, and should be taken as soon as possible whenever this diagnosis is suggested by the patient's condition.

The after-care of patients with cerebral injuries is of great importance in preventing disagreeable sequelae (headache, dizziness on change of posture, etc) or the development of chronic invalidism. The seriousness of any damage to the nervous system should not be minimized, but care must be taken not to overemphasize minor injuries. The average patient has distorted ideas regarding the disastrous effect of "injury to the brain" They should be reassured and, if circumstances warrant, should be told that there has been no significant damage to their brain and that they will be perfectly normal within a short while. A patient with a minor concussion should be allowed out of bed in 48 to 72 hours, and discharged from the hospital within 5 days He should be encouraged to return gradually to his usual activities after a few days' rest. A longer period of hospitalization and convalescence at home will be necessary for patients who have suffered more severe brain injuries Such patients should not be allowed out of bed until the cerebrospinal fluid pressure has been kept at a normal level for several days. They should be discharged from the hospital when they can walk up and down two flights of stairs without difficulty It is necessary *that the patient know that he is able to perform such activity in* [illegible] schedule [illegible] with moderately severe injuries should return to part-time work in 3 to 4 weeks after discharge from the hospital, and to full-time work within 3 months *The period of convalescence should be 3 to 6 weeks*

longer for the most severe injuries Specific directions should be given as to general hygiene and the avoidance of overmedication Alcohol should be strictly avoided.

COMPLICATIONS OF HEAD INJURY REQUIRING SURGICAL TREATMENT

Extradural Hemorrhage. The diagnosis of extradural hemorrhage is usually not difficult when the classic sequence of events occurs, i e, an injury to the head accompanied by coma followed by a recovery of consciousness and a subsequent lapse into coma coincidental with the development of a hemiplegia The diagnosis is more firmly established if roentgenograms of the skull show a fracture line running through the groove of the middle meningeal artery or one of the larger cerebral venous sinuses It must be remembered that this classic picture is only present in a little more than half of the cases For example, the brain may be so injured as to prevent the return of consciousness before the symptoms of the extradural hemorrhage are superimposed. Extradural hemorrhage is treated by immediate operation, with evacuation of the clot and ligation of the bleeding vessel A complete cure can be obtained if operation is performed speedily. Undue delay will almost invariably result in death.

Subdural Hematoma Signs and symptoms of subdural hematoma may develop in the period immediately following a head injury or they may be delayed for several months. The diagnosis of an acute subdural hematoma is often extremely difficult. It should be suspected in all patients who do not respond to routine treatment, especially if focal neurologic symptoms (hemiplegia, for example) develop or if there are alternating periods of stupor and consciousness. A shift of the pineal gland on the roentgenograms of the skull and changes in the electroencephalogram may help in the diagnosis If the presence of a subdural hematoma is suspected, small trephine holes should be made in the temporal region on both sides. When a hematoma is present, it can be evacuated by suction.

The symptoms of chronic subdural hematoma are those of an expanding intracranial lesion This diagnosis should be entertained whenever such symptoms (headache, focal neurologic signs, coma, choked disk) develop within a few weeks or months after a head

injury. Occasionally, the history of a head trauma may be lacking because the original injury was slight or the patient was intoxicated at the time and had no knowledge of the injury. Roentgenograms of the skull and the electroencephalogram are sometimes of diagnostic aid but the diagnosis can be excluded only by making small bur-hole openings in the temporal region Both sides should be explored, since hematomas are prone to give homolateral signs.

Meningitis. The diagnosis of meningitis following fracture of the skull is established by the development of an inflammatory reaction in the cerebrospinal fluid, with the presence of organisms. Meningitis following head injury may be caused by any of the pathogenic bacteria, and the treatment is the same as that previously outlined. If the meningitis is secondary to a fracture through the cribriform plate and there is persistent leakage of cerebrospinal fluid into the nose, operation for repair of the defect in the dura will have to be performed after the meningitis is cured or there will almost certainly be a recurrence

Brain Abscess Brain abscess secondary to fracture of the skull is treated by chemotherapy and surgery in a manner similar to that for abscesses from other sources.

Infected Scalp Wounds or Osteomyelitis of the Skull. These complications of head injury should be treated by drainage and excision of the infected tissues.

Aerocele The presence of air in the cranial cavity is indicative of a communication between the nasal cavities and the intracranial spaces as a result of tears in the dura The air may disappear spontaneously. If it does not, the tear in the dura should be repaired surgically, to prevent the development of meningitis.

Compound Fractures of the Skull Compound fractures of the skull are diagnosed by palpation of the skull through the lacerated scalp and should be treated by surgical debridement as soon as the condition of the patient allows Special technical details must be followed if the fracture extends into the frontal sinus Simple depressed fractures should be elevated There is usually no great need for haste in performing this operation, unless the depressed fragment is large and is injuring vital areas of the brain

Arteriovenous Aneurysms A fistula between the carotid artery and the cavernous sinus may result from traumatic injury to the carotid artery. The diagnosis is made on the complaint of a roaring

noise in the head and the presence of proptosis of the eyeball, chemosis of the conjunctiva and lids, and a loud murmur synchronous with the pulse. The treatment is ligation of the internal and common carotid artery on the side of the fistula. Before the operation is performed, the patient should be trained to compress his carotid artery for increasingly longer periods until it has been shown that a sufficient collateral circulation is present.

Cranial Nerve Paralysis. Any of the cranial nerves may be damaged by trauma to the head. Those most commonly injured are the first, seventh, and eighth nerves. The fibers of the olfactory bulb may be lacerated or the filaments of the nerve torn from the cribriform plate, producing permanent anosmia and impairment of taste for foods whose flavors depend upon the presence of volatile substances (coffee, tea, etc.). Fractures in the region of the mastoid may result in damage to the facial or auditory nerves. Paralysis of these nerves may be transient or complete. There is no treatment which will assure return of function to damaged cranial nerves. Facial paralysis can be alleviated by anastomosis of the peripheral end of the facial nerve with the central end of the spinal accessory nerve.

Trauma to the Spine

The spinal cord or roots of the cauda equina may be injured by perforating missiles, by traumatic hemorrhages, or by compression as a result of fracture dislocation of the spine. The symptoms depend on the location and extent of the lesion, and are usually a combination of paralysis and sensory loss below the level of the lesion, with loss of control of the bladder and rectum. In partial lesions of the spinal cord, the reflexes may be increased, but with severe lesions they are absent during the period of spinal shock. This period may last for only a few days or it may be prolonged for weeks or many months if infection of the bladder or bed sores develop. Traumatic hemorrhage into the substance of the cord (hematomyelia) usually gives rise to signs of a complete transverse myelitis at the onset. There will be improvement with absorption of the blood, but a varying degree of permanent disability, in the nature of weakness and atrophy of muscles at the site of the lesion and a spastic weakness of the lower extremities with or without impairment of sensation, will usually remain. The degree of recovery following injury

to the spinal cord by perforating missiles or by compression from fracture dislocations depends on the severity of the injury and the promptness of treatment.

Patients with injuries of the spine should not be moved until adequate facilities are available to insure complete immobility of the spine. Flexion of the spine should be absolutely avoided and the patient carried only in the prone position on a firm support with traction on the neck. If the injury is in the cervical region, the neck should be kept in extension, and traction maintained by the application of suitable apparatus as soon as the patient enters the hospital Fractures of the thoracic or lumbar region can be immobilized by the application of suitable casts. Diagnostic procedures that require movement of the patient should be avoided as far as possible. Roentgenograms of the spine should be taken and lumbar puncture should be performed to determine whether the symptoms are due to a compression of the cord by a fracture dislocation or whether they are the result of hemorrhage into the cord. The presence of complete or incomplete dynamic block is evidence in favor of cord compression, and is an indication for laminectomy to relieve this compression

In the first few weeks or months after injury to the spine, the care of the bladder and the skin are of paramount importance Bed sores can be avoided only by keeping the bed scrupulously clean and frequent shifting of the patient The use of the constant drainage apparatus for the bladder will prevent soiling of the bed and maceration of the skin by urine. Equally important, it will help to prevent the otherwise almost inevitable cystitis and ascending urinary infection, and when properly regulated will hasten the return of function in the bladder In this stage of recovery, physiotherapy, massage, passive movements, and muscle training are necessary to obtain the maximum return of function in partially paralyzed members.

References

1 Merritt, H H · Medical progress· Neurology, New England J Med *231*, 230, 1944.

2. Merritt, H. H Medical progress· Neurology, New England J Med *233*, 272, 1945.

3a. Sweet, L. K., Dumoff, S E, Dowling, H. F, and Leper, M. H · Treatment of pneumoccic meningitis with penicillin J. A M. A *127*, 263, 1945

3b Newmann, C. A, Heilbrunn, G, and Youmans, G P · Experiments in the treatment of dementia paralytica with penicillin J A M. A. *128*, 433, 1945.

4 Smith, M. H. D, Wilson, P. E, and Hodes, H L · Influenzal meningitis J. A M A *130*, 331, 1946

5a Nicholson, W. M, and Anderson, W. B : Penicillin in the treatment of cavernous sinus thrombophlebitis J. A M. A *126*, 12, 1944

5b. Goodhill, V.: Penicillin treatment of cavernous sinus thrombosis J. A. M. A *125*, 28, 1944

6a Nelson, R A, and Duncan, L : Acute syphilitic meningitis treated with penicillin. Am J Syph, Gonor. & Ven. Dis *29*, 141, 1945

6b Stokes, J. H, Sternberg, T. H, Schwartz, W. H, Mahoney, J F, Moore, J E, and Wood, W. G, Jr.: The action of penicillin in late syphilis J A. M. A *126*, 73, 1944

6c Goldman, D. Treatment of neurosyphilis with penicillin J. A M. A. *128*, 274, 1945

7 Merritt, H H, and Putnam, T J · Sodium diphenyl hydantoinate in the treatment of convulsive disorders J. A M A *111*, 1068, 1938

8 Merritt, H H, and Putnam, T. J · Sodium diphenyl hydantoinate in the treatment of convulsive seizures, toxic symptoms and their prevention Arch Neurol & Psychiat *42*, 1053, 1939

9 Merritt, H H, and Brenner, C: Treatment of patients with epilepsy with sodium diphenyl hydantoinate (phenytoin sodium, dilantin sodium) and phenobarbital combined J Nerv & Ment Dis *96*, 245, 1942

10 Price, J C, Waelsch, H, and Putnam, T J.. *dl*-Glutamic acid hydrochloride in treatment of petit mal and psychomotor seizures J A. M A *122*, 1153, 1945

11 Lennox, W. G · The petit mal epilepsies Their treatment with tridione J A M A *129*, 1069, 1945

12 Wolff, H. G : Experimental studies on headache, transient thickening of walls of cranial arteries in relation to certain phenomena of migraine headache and action of ergotamine tartrate on thickened vessels. Arch Neurol & Psychiat *53*, 329, 1943

13. Lennox, W G : Ergonovine versus ergotamine as a terminator of migraine headaches Am J. M Sc. *195*, 458, 1938

14 Horton, B T, Peters, G A, and Blumenthal, L S : A new product in the treatment of migraine: A preliminary report Proc Staff Meet Mayo Clin *20*, 241, 1945

15 Furstenburg, A C, Lashmet, F. H, and Lathrop, F Ménière's symptom complex Medical treatment Ann Otol, Rhinol & Laryng *43*, 1035, 1934

16 Talbott, J H, and Brown, M. R · Ménière's syndrome; acid-base constituents of blood, treatment with potassium chloride J A. M. A *114*, 125, 1940

17. Atkinson, M. Observations on the etiology and treatment of Ménière's syndrome J A M A. *116*, 1753, 1941

18 Prinzmetal, M., and Bloomberg, W.: The use of benzedrine for the treatment of narcolepsy J A M A *105*, 2051, 1935.
19a Ferrebee, J W, Atchley, D. W, and Loeb, R F.: A study of the electrolyte physiology in a case of familial periodic paralysis J Clin Investigation *17*, 504, 1938
19b Pudenz, R H, McIntosh, J F, and McEachern, D: Role of potassium in familial periodic paralysis J A M. A *111*, 2253, 1938
20 Herrington, M S: Successful treatment of two cases of familial periodic paralysis with potassium citrate, J A M A *108*, 1339, 1937
21a Viets, H R, and Mitchell, R S. The prostigmin test in myasthenia gravis New England J Med *215*, 1064, 1936
21b Viets, H R, and Schwab, R S Prostigmin in the diagnosis of myasthenia gravis New England J Med *213*, 1280, 1935.
22 Viets, H. R Myasthenia gravis J A. M A *127*, 1089, 1945
23 Blalock, A Thymectomy in treatment of myasthenia gravis J Thoracic Surg *13*, 316, 1944
24 Burman, M S Clinical experiences with some curare preparations J Pharmacol & Exper Therap *69*, 143, 1940
25 Burman, M S. Therapeutic use of curare and erythroidine hydrochloride for spastic and dystonic states Arch Neurol & Psychiat *41*, 307, 1939
26 Meyers, R: The modification of alternating tremors, rigidity and festination by surgery of the basal ganglia A Research Nerv. & Ment. Dis Proc (1940) *21*, 602, 1942
27 Wolf, G A, Jr, Goodell, A, and Wolff, H G Prognosis of subarachnoid hemorrhage J A M A *129*, 715, 1945

AUTHOR INDEX

E

L

R

S

T

Y

Z

SUBJECT INDEX

A

D

T

T

Subject Index

CUMULATIVE INDEX, VOLUMES I–III

Author Index